The Journal of Laboratory and Clinical Medicine

VOL. IX ST. LOUIS, MO., FEBRUARY, 1924 NO. 5

CLINICAL AND EXPERIMENTAL

THE RELATIONSHIP OF CHRONIC ENDOCERVICITIS TO FOCAL INFECTION WITH SPECIAL REFERENCE TO CHRONIC ARTHRITIS*

BY LAURA M. MOENCH, M.D., FELLOW IN MEDICINE, THE MAYO FOUNDATION, ROCHESTER, MINNESOTA

SINCE the widespread appreciation of the significance of chronic foci of infection in disease, as suggested by Billings and developed by the experimental work of Rosenow, clinicians have carried on the routine examination of tonsils, teeth, accessory sinuses, and the urinary tract, with the expectation of eradicating factors having a direct bearing on disease. While such tissues are now definitely recognized as chronic foci, there are also tissues capable of harboring infection, but their function, as agents of disease, still remain uncertain. In this group may be enumerated the infections of the lymphatic tissue of the appendix, chronic salpingitis, phlebitis, cholecystitis, chronic vegetative endocarditis, and finally, endocervicitis. It is my purpose to discuss the possibility of a definite relationship between chronic infection of the cervix uteri and morbidity in general, and to indicate an association with disease influenced by the presence of such chronic foci.

Until the bacteriology of endocervicitis received attention, a leucorrheal discharge was considered the extension of a general infective process involving the myometrium of the uterus, rather than itself a clinical entity. In 1888, however, Winter demonstrated, in an examination of the fallopian tubes and uteri removed by abdominal section, that above the cervix the uterus was practically free from bacteria, placing the normal boundary between the germ-bearing and bacteria-free portions of the tract at the internal os. Stroganoff, Menge and Walthard, in 1895, showed the bacterial

*Abridgment of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine, July, 1923.

Received for publication, September, 1923.

boundary to be even lower, to correspond with the external os. Menge and Krönig, in 1897, published observations made on material aspirated from the uterus, and concluded that bacteria did not live in the uterine cavity. Still more recently it has been questioned whether, even in cases of so-called endometritis, the uterus itself is infected. Evidence of such infection, from a pathologic standpoint, was found to be relatively infrequent by Strong, who examined curettings from all types of cases over a period of six years. Hitschmann and Adler, in 1907, asserted that most cases classified as endometritis are devoid of histologic signs of infection. Curtis, in 1918, made a combined bacteriologic and histologic study of 118 uteri removed by abdominal section, on the basis of which he concluded "that infection of the uterus commonly called 'chronic endometritis' is practically to be ruled out as a clinical entity."

The histology and function of the cervix, however, obviously render it more susceptible to the incidence and harboring of infection. Whereas the endometrium of the uterus is continually undergoing the active hyperplasia and desquamation attending menstruation, which act as a recurring physiologic catharsis, the function of the cervix requires that it remain a stationary structure, acting merely as a passive communicating channel. The cervix is, moreover, lined with mucus-secreting glands, whereas from the endometrium of the body of the uterus there is practically no secretion, and the racemose character of the cervical glands, the tufts of which penetrate deeply into the myometrium, renders them peculiarly liable to the perpetuation of infection. By their invagination into deeper structures they tend, furthermore, to the setting up of a chronic inflammatory reaction within the myometrium and interstitial tissues. As Polak observes, "sooner or later the deeper structures are always involved, producing a cervical metritis." The plugging of the ducts with the products of inflammation and with quantities of mucus results in the development of retention cysts, a further barrier to drainage and an added factor in the bulk and congestion of the cervical tissue. As the picture of infiltration and congestion advances, the cervical mucous membrane becomes hyperplastic, swollen and everted, producing the co-called erosion of the external os. "Microscopically," as Matthews described, "in the musculature nearest the cervical mucosa may be found small inflammatory foci or even small multiple abscess formations . . . The clinical course is slow and insidious and shows little or no tendency towards a spontaneous cure."

Infection of the cervix has been widely recognized as potentially of more than local significance. Polak states, "Leopold's description of the lymphatic distribution in the uterus suggests the far-reaching pathology which may result from a chronic cervical infection." Owing to the lymphatic drainage of the posterior surface of the cervix, Matthews says "Chronic endocervicitis is always attended by some degree of posterior cellulitis." Sturmdorf, Matthews and others recognize in the distribution of the cervical lymphatics the basis for an extensive ascending infection of the pelvic structures, and the significance of chronic cervicitis to the system as a whole is suggested

by Sturmdorf in his epigrammatic likening of the cervix to the "tonsil" of the uterus. "This pathogenic parallel," he says, "finds its applicability when chronic endocervicitis is recognized as a primary infectious focus and its systemic symptoms as toxic manifestations."

Origin of the Infection.—In dealing with endocervicitis as a primary focus it is necessary to differentiate this affection from discharges originating higher, or from the vaginal structures. The uterus, then, except in the presence of pyometritis, can be ruled out as a source of discharge (Curtis). Leucorrheas originating from chronic pelvic inflammatory disease may be eliminated on the basis of the history and physical findings. The congestion attending uterine displacements, tumor of the pelvis, both intrauterine and extrauterine, varicosities of the pelvic veins and other forms of systemic edema, may give rise to an increase of the normal mucoid discharge. Finally, the vagina must be eliminated as a source of the leucorrhea, both with regard

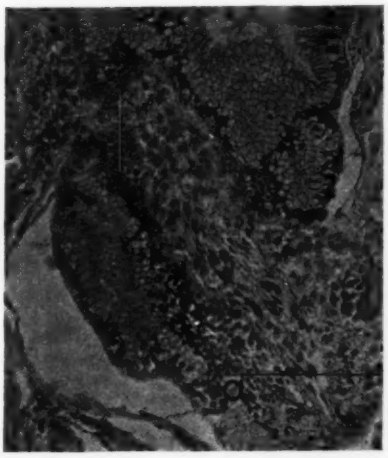

Fig. 1.—Chronic endocervicitis. *a*. Products of inflammation breaking through the mucous membrane. X 120.

to chronic vaginitis and infection of Skene's ducts. The association of a cervical discharge with a hyperplastic, congested and edematous cervix, hyperemic and frequently eroded, with or without laceration, is sufficient to establish the lesion as primary in the cervix. Such an infection may occur as a result of an increased susceptibility to infection from a passive congestion, as occurs in the presence of pelvic tumors, intrauterine or extrauterine, as result of conditions attending subinvolution of the uterus, lacerations of the cervix, or, of a general lowering in systemic resistance as from chronic illness and anemia, and finally, as the end result of a gonorrheal infection which, as Curtis suggests, "prepares the soil for the invasion of more chronic organisms" (Fig. 1).

Flora of the Genital Tract.—The bacteriology of leucorrhea had received but scant attention prior to the classification of bacteria of the genital tract by Curtis, in 1914. The work of Döderlein, in 1892, Menge and Krönig, in 1897, Wegelius, in 1909, and Heurlin, in 1910, in all yielded no more than

a description of the vaginal bacillus, and a rough classification of organisms into aërobic and anaërobic forms. In 1914, Curtis published a study of the etiology and bacteriology of endocervicitis in a series of seventy-five cases, in thirty-five of which there was profuse leucorrhea. The bacteriology was found to vary with the character of the discharge, the mucoid types presenting a picture which approached the normal. In purulent discharges, however, appeared the greatest variations in floral types. The greater number of bacteria in leucorrheal discharges were anaërobic in nature, whereas cultures obtained from the vulvar region, or high vaginal cultures from patients who were subject to frequent douching, contained more aërobes, notably colon bacilli, staphylococci and pseudodiphtheria bacilli. The predominating organisms were gram negative, anaërobic bacilli of varying morphology and cultural characteristics. The gonococcus was encountered frequently enough to reveal its importance as the primary cause of subsequently developing leucorrhea. The colon bacillus, *Bacillus bifidus* and pseudodiphtheria bacilli were met with inconstantly. Finally staphylococci and streptococci, both anaërobic and aërobic, are described, and interest centered about these organisms has developed in the following study.

BACTERIOLOGIC AND ANIMAL STUDIES

At the suggestion of Dr. Leda J. Stacy, of the section on gynecology of the Mayo Clinic, an attempt was made to further the conception of the cervix as a focus by a study of the bacteriology of cervicitis, supplemented by an intensive study of the pathologic conditions produced by this flora on animal inoculation. Two series of cases were chosen, one in which evidence was lacking of secondary disease, and a second series referred from the section on orthopedic surgery, and the section on ophthalmology, consisting of conditions recognized as associated with a focus or foci of infection, but in which all foci other than the pelvis had been eliminated.

A series of eighty-two miscellaneous cases with leucorrhea were, therefore, studied bacteriologically, thirty-eight strains of which were used for animal inoculation. The following technic was employed. After thoroughly removing the mucus and pus from the external os, cultures were made from the cervical canal with sterile swabs, or the material aspirated from the cervix through Pasteur pipettes for saline suspension. Anaërobic cultures of the discharge were made on blood agar slant and plates by the pyrogallic acid method, aërobic blood agar, plain agar, and litmus lactose agar enriched with casein digest, were used for the study of the types of organisms present, and in all cases dextrose-brain-broth was employed for the major portion of the work, as offering both aërobic and anaërobic conditions of growth in a fluid medium. Preliminary films made directly from the cervical discharge were studied routinely.

Cervical Flora.—These organisms were found to correspond closely with the classification detailed by Curtis. The large majority were found to require anaërobic conditions of growth, the anaërobic blood agar slants showing an elevated and rich growth, "roughly beaded" and "fairly well con-

fined," whereas the aërobic plates yielded, in comparison, very poor growth. Gram-negative bacilli with an admixture of diphtheroid, pleomorphic gram positive bacilli comprised a large proportion of the anaërobic organisms with a varying percentage of anaërobic streptococci, the latter appearing on blood as indifferent colonies consisting of diplococci, or as short chains, often taking an irregular stain. In fluid mediums these chains assumed a large number of elements, and on subculture tended to lose their irregular staining and to become aërobic (Fig. 2). The colon bacillus and other fecal organisms were encountered only when douching or previous examination had introduced vulvar organisms. The streptococcus was a constant, conspicuous organism in films made directly from the leucorrheal discharge, and grew in great luxuriance in the brain-broth cultures. The aërobic streptococcus usually encountered appeared as a lanceolate diplococcus with but slight tendency to grow in chain formation (Fig. 3). An aërobic chain-former was, however, not infrequently recovered (Fig. 4). Staphylococci were common. A hemolytic streptococcus was twice isolated, in both instances in association with evidence of local virulence, ulcer of the vagina in one case, thought to be secondary to the cervical discharge, being present. The number and variety of the organisms, and the percentage of streptococci, varied with the character of the discharge, the mucoid discharges containing relatively few organisms, the purulent, and especially, the seropurulent discharges, being rich in streptococci.

Fig. 2.—Anaërobic streptococcus. Tissue culture of deep cervical structures. High specificity for joints. X 1000.

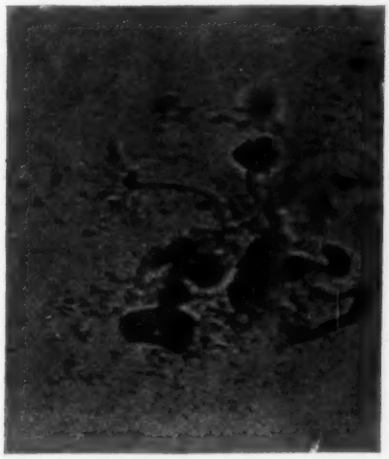

Fig. 3.—Film from leucorrheal discharge showing gram-positive diplococci. X 1000.

Technic for Animal Inoculation.—Pathogenicity was directly determined by injecting, intravenously, suspensions of the leucorrheal material in saline solution, when such a suspension could be secured sufficiently free of fecal contaminants, and parallel cultures plated on anaërobic and aërobic blood agar to determine the character of viable organisms. A second series of animals was injected with from 2.5 to 3 c.c. of an eighteen-hour growth of dextrose-brain-broth culture. The animals in both series were chloroformed

and examined in from three to eight days. Cultures were made at necropsy in dextrose-brain-broth and on blood agar from the kidneys, blood, spleen, bile, pericardial fluid, adrenals and joints, and when lesions were found the tissue was preserved for histologic study. Of the 216 animals used, eighteen died within the first two days from septicemia, usually from fecal contaminants. The surviving animals appeared inactive with increased respiration for the first twenty-four hours, following which they continued sluggish for a week or ten days. Many were unable to use the joints of the extremities. The pathologic conditions revealed at necropsy are given in Table I.

Streptococci not Eliminated but Localized in the Tissues.—Cultures from tissues at necropsy revealed the fact that the streptococci recovered from these tissues definitely outnumbered the other organisms injected. Mixed cultures, however, were obtained when the animal was examined within two days after injection, as a rule, from the spleen, kidneys, urine and blood.

Fig. 4.—Aërobic diplostreptococcus. Most common organism recovered in pure culture. X 1000.

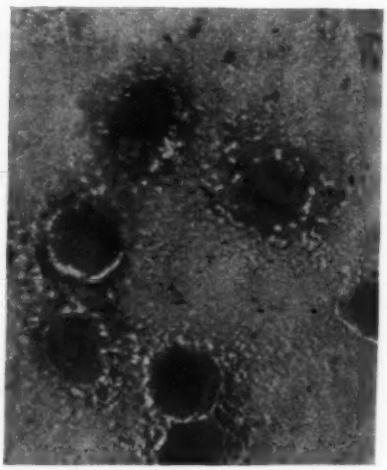

Fig. 5.—Purulent exudate from knee-joint of rabbit, showing pus cells and diplococci. X 1000.

From the joints, even when gross lesions were not apparent, other than a serous effusion, streptococci were recovered in pure culture in heavy growth before cultures from other tissues showed signs of bacterial development (Fig. 5). A purulent arthritis was indeed often the only lesion encountered. With regard to gross pathology, cholecystitis occurred next in frequency, with muscular hemorrhages third. Pericardial effusion from which the organism was recovered was occasionally encountered. Lesions in the kidneys were present infrequently, and in no case was hemorrhage of the mucous membrane of the stomach, or appendix encountered. Early isolation of the organism from the original conditions of growth seemed definitely to affect the tendency to joint localization. Saline suspensions, when sufficiently free from fecal contamination to be given in heavy suspension intravenously, showed greater tendency to localize in the joints than did eighteen-hour brain-broth cultures, in spite of numerically greater concentration of organisms in the latter. Early brain-broth cultures showed higher apparent

specificity for joints than did older cultures. Certain strains from the first series retained the tendency to localize in joints through two animal passages; in the latter series of arthritis cases, several strains retained this characteristic through three and four animal passages.

Similarity of Organisms to Diplococci Described in Association with Arthritis, and Comparison with the Pathogenicity of Streptococci from Dental and Tonsillar Foci.—A resemblance was noted of the streptococci recovered, to the organism described by Rosenow, in 1913, which he obtained from glands draining arthritic joints, in its marked affinity for joints when

TABLE I

RESULTS OF ANIMAL INOCULATION IN UNSELECTED CASES

	STRAINS OF STREPTOCOCCI	ANIMALS INJECTED	ANIMALS WITH LESIONS OR POSITIVE CULTURES IN								
			MUSCLE	JOINTS	GALL-BLADDER	SPLEEN	ADRENALS	KIDNEYS	BLADDER	PERICARDIUM	BLOOD
On isolation	38	86	8	20	15	18	5	27	4	4	20
On second animal passage	8	14	0	1	1	5	0	7	9	0	5
Per cent		100	8	21	16	23	5	34	13	4	25

TABLE II

RESULTS OF ANIMAL INOCULATION IN CASES OF ARTHRITIS

	STRAINS OF STREPTOCOCCI	ANIMALS INJECTED	ANIMALS WITH LESIONS OR POSITIVE CULTURES IN						
			MUSCLES	JOINTS	GALL-BLADDER	SPLEEN	KIDNEYS	URINE	BLOOD
On isolation	16	73	8	43	15	13	31	13	8
On second animal passage	7	31	2	13	2	1	11	8	3
On third animal passage	3	10	1	4	3	1	2	2	0
On fourth animal passage	1	2	0	1	1	0	1	0	0
Per cent		116	9	52.5	19	12.9	37	20	9

first isolated and in its preference for anaërobic growth. An organism recovered from cases of rheumatic fever, similar morphologically, was described by Poynton and Paine in 1910. It was thought, therefore, that there might prove to be an etiologic relationship between the cervical streptococci, as described, and certain cases of subacute or chronic arthritis. Curtis believed that the most important cervical organisms were the gram-positive diplococci, and in directing attention to the possible focal relationship of the cervix, remarked that chronic arthritis had been noted with unexpected frequency in patients without other demonstrable foci.

In comparing this series of cases with that of Meisser, whose animals were injected with streptococci recovered from dental foci, the percentage of joint lesions stands in the relation of twenty-one as against eight of

animals inoculated, and in a series of rabbits inoculated with streptococci obtained from tonsils of patients without arthritis, this percentage of animals inoculated appeared as 25 per cent (Nakamura). With respect to joint specificity, therefore, the cervical streptococci would appear to fall within the classification recognized as belonging to the focal group.

In accordance with Rosenow's theory of the specific localization of streptococci, it was decided to select a series of patients with subacute or definitely progressing chronic arthritis in whom other foci of infection had been eliminated, but whose symptoms bore evidence of the continued activity of a focus of infection within the body. Twelve patients of this type were selected. The technic employed was the same as that used in the first series, and the result of tissue localization in the second series of animals is summarized in Table II. A definite increase in specificity for joint tissues was apparent, with a slight associated elevation of virulence of the streptococci for all tissues. The tendency to joint localization was retained over three and four animal passages, as against two in the first series.

Factors Governing Virulence and Specificity of the Organism.—As was observed in the first series, the tendency to localize in joints appeared to vary directly with the age of the cultures, saline suspensions showing early a specific localization in joint tissues, whereas older cultures in brain-broth of thirty-six to forty-eight hours were relatively inert in this respect. In view of this apparent dependence of the organism for virulence on original conditions of growth, an effort was made to acquire a strain directly from the deeper structures of an infected cervix amputated as a possible focus in a case of rapidly progressing arthritis. As it seemed possible to predict, the strain recovered from this source both in saline filtrate of macerated tissue and in eighteen-hour broth culture, showed a striking tendency to localize in joint tissue. Ninety-eight per cent of a series of rabbits inoculated with this strain developed a purulent arthritic exudate, as against 54 per cent in a series inoculated with organisms obtained from the leucorrheal discharge. This strain, preserved for several months in a saline glycerine suspension, lost its characteristic long-chain formation, breaking into shorter chains, and finally assumed a lanceolate diplococcal form, identical morphologically and in reactions on sugar mediums, with the aërobic forms constantly recovered from leucorrheal discharges. The ability of cervical streptococci to change, in a parallel manner, their biologic characteristics, was indicated by the tendency of the anaërobic forms to become aërobic in old cultures and in rapidly made subcultures. Five strains strictly anaërobic in primary culture, freshly isolated and plated on aërobic blood agar, failed to show evidence of growth. Rapid subculture of these strains was then undertaken, transferring by means of capillary Pasteur pipettes, the rapidly growing cultures from the anaërobic portions of the dextrose-brain-broth tube when the first haze of growth appeared over the tissue at the butt of the tube, into a second tube of dextrose-brain-broth, each new subculture being simultaneously plated on aërobic blood agar plates, and on anaërobic plates, to insure the continued identity of the strain. In the fourth, sixth, seventh and ninth

subculture these strains were found to have acquired aërobic properties, growing richly on aërobic plates. The aërobic strains thus produced showed reactions on sugar mediums identical with those given by the frankly aërobic streptococci obtained from the leucorrheal discharge. On the basis of this experiment it was thought valid to regard the aërobic strains as modified forms of the more highly virulent and more definitely specific anaërobic streptococci deeply embedded in the submucous muscular tissues of the infected cervix. Such an explanation would account for the marked specificity for joints (98 per cent) displayed by the anaërobic streptococcus obtained from the deeper tissues, in comparison with the definite but lower percentage of localization (54 per cent) which occurred with the aërobic strains obtained from the leucorrheal discharge of the same patient. On the basis of this observation it was thought probable that the percentage localization in joints

TABLE III

FERMENTATION REACTIONS OF CERVICAL STREPTOCOCCI

STRAIN	BLOOD AGAR	MILK	GELATIN	DEXTROSE	SACCHAROSE	INULIN	RAFFINOSE	MANNITE	LACTOSE	SALICIN
1	Hemolytic	Acid		1	+	0	0	+	+	++
2	Indifferent			+	+	0	±	0	+	++
3	Indifferent			+	+	0	±	0	+	++
4	Indifferent			+	+	0	±	+	+	++
5	Indifferent			+	+	0	±	0	+	++
6	Indifferent	Acid	?	+	+	0	±	0	+	++
7	Indifferent	Acid	?	+	+	0	±	+	+	++
8	Indifferent	Acid	?	+	+	0	±	0	+	++
9	Indifferent	0	0	+	+	0	±	0	+	++
10	Indifferent	Acid	?	+	+	0	±	0	+	++
11	Indifferent	Acid	0	+	+	0	±	+	+	++
12	Indifferent	Acid	0	+	+	0	±	0	+	++
13	Indifferent	Acid		+	+	0	±	0	+	++
14	Indifferent	Acid		+	+	0	±	±+	+	++
15	Indifferent	Acid		+	+	0	±	+	+	++
16	Indifferent	Acid		+	+	0	±	±+	+	++
17	Indifferent	0		+	+	0	±	±	+	++
18	Indifferent			+	+	0	±	+	+	++

obtained in the entire second series, through the use of cultures consisting of leucorrheal streptococcal forms, might not represent the actual pathologic potentiality of the organism harbored within the more deeply infected cervical tissues.

Fermentation reactions of a series of leucorrheal strains of cervical streptococci revealed a fairly high degree of uniformity. Except for slight variation in their ability to ferment mannite, the strains were similar in their reactions on sugar mediums. The strains thus studied are presented in Table III.

SEROLOGIC AND IMMUNOLOGIC STUDIES

Serologic Evidence of Antibodies Against the Streptococci in the Cervix in a Case of Arthritis with Acute Exacerbations: Antigenic Properties of Cervical Streptococci.—In the majority of chronic cases of arthritis there is slight evidence of general reaction to the infecting organism. However, an occasional case occurs in which a febrile reaction bears witness to autoinoc-

ulation. Case 12 of this series presented such an instance, and the opportunity was seized for serologic study. During the postfebrile phase of three exacerbations, a series of dilutions were made with equal proportions of a saline suspension of the strain of streptococci recovered from the deeper tissues of the amputated cervix and repeated for six days following such exacerbations. Agglutination occurred constantly with clearing in dilution of 1:200, the controls with normal human serum ranging from no change in turbidity to an indefinite flocculation.

In view of this finding it was decided to test the potential antigenic properties of this organism with a view to therapeutic application. A series of rabbits was immunized as described under the case history, by increasing doses of killed organisms at bi-weekly intervals. A similar series was simultaneously immunized with a streptococcus strain from a nonarthritic case. At the beginning of the fourth week the titer for the homologous streptococcus had risen to 1:14,000 against the blood of the immunized rabbits. There was no evidence of cross agglutination in either series. The aërobic form obtained from the leucorrheal discharge showed indefinite agglutination in the immune serum, as did also subcultures of the strain used for immunization. There was no agglutination with streptococci from other sources. Serums specific for the Rosenow streptococcus of poliomyelitis, influenza or encephalitis produced no agglutination with the "arthritic" strain of cervical streptococci. The homologous serum, both from the human patient and from the immunized rabbits, showed definite antibody reaction, and in view of this observation the possibility of raising the patient's resistance to the infecting organism seemed assured.

Variation in Response to Vaccines in Human Subjects.—The reaction of different types of cases to vaccines of cervical streptococci showed some variation. The strain recovered from the patient in Case 12 produced marked reaction in this patient with delay of a return to normal, and with evidence of severe systemic tax. It was discontinued because of apprehension of reaction. In a more chronic form of the disease a mild reaction was the rule. In general, a gradual improvement of symptoms occurred, even when clinical evidence indicated that the cervical focus had not been entirely removed, but in these instances there was a tendency to periodic relapse. Three cases referred from the section on ophthalmology, two of episcleritis and one of choroiditis, without other ascertainable foci, reacted with moderately severe general and local symptoms; one patient remained in bed for twenty-four hours. All three of the patients had marked exacerbation of the condition of the eye for a period of several days, followed by a rapid regression of the pain and inflammation. In one case (of eighteen months' standing) complete healing occurred within ten days following vaccine, and in one there has been definite improvement to date, a period of ten days. The third patient improved definitely, but left the clinic before the outcome of the infection could be determined. With the apparent evidence of the high antigenic properties of certain strains of the cervical streptococcus it would seem, therefore, that following complete eradication of the focus we may

possibly be justified in looking to vaccines of the more specific form of this organism for therapeutic aid in the treatment of chronic arthritis, and possibly in other chronic progressing conditions in which the cervix appears to exist as an active focus of infection.

REPORT OF CASES

CASE 1 (A351819).—Mrs. T. K., aged thirty years, came to the Mayo Clinic March 8, 1921, having been referred by her family physician for her chronic arthritis involving the right knee, the tarsometatarsal joints of both feet, the left acromioclavicular joint and, to a less degree, the lumbar spine. She was in bed for nine weeks at the onset of the disease in 1916, during which time there were swelling and pain in the right knee and foot. Following this she was comparatively free from trouble until December, 1920, when she developed tenderness and pain in the lumbar region, radiating down the thigh to the heels, with marked swelling and pain in the left acromioclavicular joint. At the same time she noticed that a leucorrheal discharge, which had previously not troubled her greatly, had become profuse.

Examination.—The physical examination was negative except for tenderness, graded 2+, over the sacroiliac juncture, and medial aspect of the foot. There were also marked tenderness and slight swelling over the acromioclavicular juncture. Roentgenograms of the joints were negative. The tonsils had been cleanly removed. Two teeth showed apical infection and were removed while the patient was at the clinic. Pelvic examination revealed a boggy edematous cervix, somewhat lacerated, with a mucopurulent discharge. Bacteriologic examination revealed a predominating streptococcus in eighteen-hour culture, growing in diplo-arrangement and in short chains.

Animal Inoculation.—Six animals were injected, three with a saline suspension, of which two developed an acute purulent arthritis; three with an eighteen-hour culture, of which one developed joint lesions. The streptococcus was recovered in pure culture from the joints. On second animal passage one of four animals developed lesions of the joints. Culture from two infected teeth revealed a predominating green-producing streptococcus. Two animals injected with 5 c.c. of an eighteen-hour culture failed to develop lesions of the joints.

Subsequent Course.—On the patient's dismissal autogenous vaccine and douches were advised. The vaccine given was associated with slight symptomatic improvement over a period of several months, following which the pain returned in its original intensity. The patient was advised to return for more radical treatment of the cervical infection, but failed to do so. A communication from her physician in January, 1923, stated that apparent cure of the infection had been accomplished by the use of protargol douches. Since the disappearance of the cervical discharge all manifestations of arthritis had disappeared and the patient had been entirely free from symptoms.

CASE 2 (A356621).—Miss C. G., aged twenty-four, came to the clinic in April, 1921, because of progressing arthritis, which she had first noticed in 1916. The onset had been gradual, but without remission except for a period of several months following rest and treatment. During the winter preceding her admission to the clinic the right knee became acutely swollen and painful, and the left knee and ankle so painful that she was confined to a wheel-chair. She complained of pain, swelling and stiffness in her knees, ankles and finger joints.

Examination.—The knees were found to be markedly hypertrophied, extension and flexion being definitely limited. There was slight atrophy of the muscles of the calf, and limitation and deformity of the right elbow, fingers and wrist of the right hand. The routine examination for foci was unproductive, except for one tooth doubtfully infected, which was removed. Roentgenograms of the joints revealed destructive arthritis with fixation of the right knee and elbow. Examination of the pelvis revealed marked, definitely purulent leucorrhea with hypertrophy and congestion of the cervix.

Animal Inoculation.—Of three animals inoculated with primary cultures, two developed lesions of the joints, from which the streptococcus was recovered in pratically pure culture. Of two receiving saline suspensions, one showed joint localization with recovery of the streptococcus. Other tissues produced no growth. Areas of myositis were present in three animals of the series. Cultures of the granuloma of the infected tooth inoculated into a series of animals did not produce lesions of the joint, although muscular hemorrhages were observed. The green-producing streptococcus was recovered from the fallopian tube of one of the animals examined, and from the degnerating embryonic sac of another, suggesting the possibility of blood-borne infection in some cases of streptococcal adnexal disease in rabbits.

CASE 3 (A357946).—Miss F. E. S., aged nineteen years, came to the Clinic in May, 1921. Eighteen months prior to admission she had noticed slight swelling of her left knee which came only during the menstrual periods and continued for several days, at intervals of twenty-eight days, for one year. Six months prior to admission she began having diffuse continuous pain in the knee, especially over the medial aspect, so that she was unable to bear her weight on the leg. During this time, for a period of three weeks, she had fever of 101°, intermittently, and was confined to bed. The right elbow became involved several weeks after the knee and caused constant pain, worse on motion. Her general condition was unsatisfactory. She complained of general muscular pain, fatigue and nervousness; her feet and hands were cold, and she perspired easily.

Examination.—Except for evidence of the present illness general examination was negative. The left knee was swollen and tender, with some local heat; extension was limited to 160° and flexion to 80°. The right knee was slightly swollen. Roentgenograms of the left knee revealed beginning arthritis, but those of the elbow and hand were negative. No focus was demonstrated in a routine examination of the nasopharynx, tonsils, sinuses and teeth. There was slight leucocytosis ranging from 10,000 to 15,600. No history of leucorrhea was obtained, the hymen was intact, but on examination with a speculum, a definite, thin, seropurulent, cervical discharge, with a narrow erosion around the external os, was observed.

Animal Inoculation.—Of eight animals inoculated with primary cultures the streptococcus was recovered from the joints in six. On second animal passage of five animals, four developed arthritis. On third passage two of four developed lesions of the joints, and on fourth animal passage one of two. The streptococcus was recovered in pure culture from all joints showing gross lesions. A slender gram-negative anaërobe was recovered in association with the streptococcus from the spleen, kidneys and urine in one rabbit on direct inoculation and from another on second animal passage. There was no evidence of this organism in cultures made from joint exudates.

Discussion.—The clinical history of this case, with an onset of periodic joint effusion coinciding with the menstrual periods for a year preceding the development of pain and local involvement, with remission during the intermenstrual phase, with activity of a pelvic infection, suggested an etiologic relationship. The cervical infection was of slight local significance, but in view of the pathogenicity of the organisms harbored for joint tissues, douches were instituted and vaccine of the streptococci administered. There was apparent symptomatic improvement for one year, when the patient had an acute relapse with fever and total disability for two and one-half months. The vaccine was then entirely discontinued. Following the acute exacerbation the patient gradually improved from September, 1922, to August, 1923. Except for a continued slight swelling of the knee joints coincident with her menstrual periods, to date the patient regards herself as free from symptoms.

CASE 4 (A344780).—Mrs. A. P. F., aged fifty-three years, came to the Clinic in October, 1921. For the preceding eight or nine years she had had attacks of pain in the right leg, beginning in the arch of the right foot, later involving the knee and the shoulder joint. At first the pain appeared to be only in the tissues around the joint, but more recently the joints themselves had become painful and stiff. All of the teeth had

been extracted, and tonsillectomy performed the summer of 1920, without noticeable benefit. There had been no acute infections. Menopause had occurred two years before and had been uneventful. The symptoms involving the joints had become markedly worse, however, since the menopause, and the presence of a slight leucorrheal discharge had been noted. There was slight constipation, but appetite and general health were good.

Examination.—Examination revealed Heberden's nodes on the second and third fingers of each hand. Roentgenograms of the joints were negative. Routine search for foci was unproductive. Tonsillar rests were removed, and animal study from tissue cultures were made without evidence of joint localization. Because of the leucorrhea noticed since the onset of the menopause the patient was referred to the section on gynecology for cultures and bacteriologic study.

Animal Inoculation.—Primary broth cultures revealed the predominating organism to be a short-chained streptococcus, and eighteen-hour cultures were injected intravenously into three rabbits. On the seventh day, one rabbit had developed acute purulent arthritis, cultures from the joint exudate being positive for the organism injected in pure culture on blood agar. The remaining two animals did not have lesions. Cultures of other tissues were negative except from the bile in one instance.

CASE 5 (A237757).—Mrs. E. M. H., aged thirty-three years, came to the Clinic in September, 1921, complaining of stiffness and pain in her fingers. The onset of the pain had occurred two months previously at which time she had first noticed a thin, purulent discharge from the vagina. She had been troubled for several years with pain in the left iliac region which had been ascribed to a sacralization of the fifth lumbar vertebra on the left side; this pain, and slight muscular aching, had been more troublesome during the last two months.

Examination.—The general examination was negative. Roentgenograms of the lumbar region revealed sacralization of the fifth lumbar vertebra on the left side. The tonsils had been cleanly removed. There was no dental focus. Examination of the pelvis demonstrated the cervix to be slightly hypertrophic and nodular, with a thin, purulent discharge. There was no adnexal thickening.

Animal Inoculation.—Two animals were inoculated with eighteen-hour cultures. Of these one developed a purulent joint lesion from which a streptococcus was recovered. Films of the joint exudate showed many pus cells and intracellular and extracellular gram positive diplococci.

CASE 6 (A351162).—Mrs. P. I. V., aged twenty-five years, came to the Clinic in February, 1921, with arthritis of the hip, right wrist and knee. She dated the onset of the condition from the puerperium following the birth of her child three years before. She was, at this time, confined to bed for eleven weeks with pain and swelling of the right knee, and with incapacity in the joint for three months. Following subsidence of pain and swelling in the knee, practically all of the large joints became involved. There had been swelling and stiffness in the joints of the fingers of both hands for the last year. Since the onset of the arthritis the patient's general health had been poor.

Examination.—The patient was markedly underweight. A loud systolic murmur at the apex and over the whole precordium, was transmitted to the axilla. The fingers were swollen and there was some limitation of motion. There was limitation of the motion of the lumbar and dorsal spines because of pain. Roentgenograms of the spine were negative. There were foci in teeth and tonsils. On pelvic examination a heavy mucopurulent discharge and a granular cervical erosion were noted. There was some evidence of an old pelvic adnexal disease, but no demonstrable activity of the process and no tenderness were elicited.

Tonsillectomy was performed at the clinic, followed by definite improvement for several weeks. At the end of this time, the dental extractions were performed at the patient's home. The patient returned in October of the same year, stating that there had been temporary improvement in the joints, but that symptoms had returned. On reexami-

nation there was little evidence of pelvic infection other than the cervicitis. Cultures were made from the mucopurulent discharge.

Animal Inoculation.—Of six animals injected with mixed cultures, three developed joint lesions. A vaccine was made from the strain recovered from this source.

Subsequent Course.—A partial amputation of the cervix was performed for the purpose of eradicating the cervical infection, and the patient returned home under the care of her physician. A vaccine of the cervical streptococci was administered at weekly intervals for a period of seven months, following which the patient experienced a marked reaction, with chills and fever, and with increased local pain for several days. During the course of this treatment the arthritic symptoms definitely subsided, but during the reaction period following each vaccination she experienced marked pelvic pain. In May, 1922, she wrote that she had discontinued the vaccine on this account, and shortly after suffered an acute exacerbation of arthritis in her hands and shoulders. A letter received in July, 1923, stated that the cervical discharge had returned, obviously a case in which surgical procedure had been insufficient to eradicate the cervical infection.

CASE 7 (A377865).—Mrs. T. T., aged forty years, came to the Clinic in November, 1921, complaining of pain in the back, and general aching and malaise. She dated the onset of her symptoms from the appearance of a leucorrheal discharge four years previously, and had undergone a curettment elsewhere because of the observed association between the leucorrhea and the arthritis. She felt that her symptoms had improved during the six months immediately following this procedure. The history of pelvic trouble, except for the leucorrhea, was negative.

Examination.—General examination and roentgenograms were negative. The condition was regarded in the section on orthopedic surgery as a low grade myositis with probable beginning spondylitis involving the lumbar and cervical spines. There was slight dental infection which was removed while the patient was at the clinic. Examination of the pelvis revealed a thin, purulent discharge from the cervix.

Animal Inoculation.—Bacteriologic examination of the discharge from the cervix revealed a predominating growth of short-chained streptococci in dextrose-brain-broth. Eighteen-hour cultures were injected into two rabbits, and examined on the sixth day. The organism was recovered in both animals from the joints, kidneys, and urine. The other tissues did not produce growth.

CASE 8 (A378537).—Mrs. R. M., aged forty-two years, came to the Clinic in November, 1921. Three years before she began noticing dull pain in the lumbar spine. There had been stiffness and pain in the right knee for an indefinite period. A profuse leucorrhea had been present for a number of years.

Examination.—There was limitation of motion in the lumbar spine. Roentgenograms revealed hypertrophic arthritis of the lumbar spine and of the right knee. Slight dental infection was eradicated while the patient was at the Clinic. Examination of the pelvis revealed a discharge, mucopurulent in character, with uterus slightly retroverted.

Animal Inoculation.—Two animals were inoculated with broth cultures and examined ten days later. One rabbit did not use the right hind leg, and purulent arthritis was discovered involving the right knee joint. The second animal had areas of myositis, but no evidence of joint involvement.

CASE 9 (A378374).—Mrs. E. G., aged thirty-four years, registered at the Clinic in November, 1921. The onset of her arthritis had occurred eight years before, following the birth of her second child. The finger joints, the elbows and shoulders, and subsequently all of the large joints of the body had become progressively involved. The joints affected were red, swollen, hot, and with a characteristic atrophic glazing of the skin. There was also some muscular atrophy. During the third and last pregnancy she had been entirely free from symptoms which, however, had returned soon after childbirth. Following the last pregnancy she had noticed a somewhat profuse leucorrheal discharge. She was conscious of definite improvement in her symptoms during the menstrual period, although for

several days prior to the period there would be a definite exacerbation of pain and swelling in the joints.

Examination.—The patient was definitely undernourished. The joints of both hands were swollen, with slight deformity, and ulnar deflexion. Practically all of the large joints were tender on manipulation. The tonsils had been cleanly removed. Roentgenograms revealed periapical infection in six teeth, which were removed. Examination of the pelvis revealed a soft, boggy cervix with a granular erosion and a mucopurulent discharge.

Animal Inoculation.—Three animals were inoculated and examined in three and four days. The streptococcus was recovered in heavy eighteen-hour growth from the joints. Cultures of all other organs, except in one animal, from the spleen and kidney, were negative.

CASE 10 (A395969).—Miss M. R., aged thirty-two years, registered at the Clinic in June, 1922. Pain had first been noticed five years previously in the joints of the toes of the right foot. A year later the ankles became similarly involved. In August, 1920, the joints of the hands became red, swollen and painful. Nonspecific protein had been given intravenously without benefit to the joints, and slight anaphylactic symptoms had developed. Except for a slight leucorrhea of unknown duration, there was no history of pelvic trouble.

Examination.—The joints involved in the arthritic process were the middle phalangeal of the fourth finger of the right hand. Ankylosis of the terminal joint was present with slight boggy edema around the other joints of both hands. Roentgenograms revealed slight bony change. The tonsils had been cleanly removed elsewhere. Routine search for foci was unproductive. Examination of the pelvis revealed a definite, creamy, cervical discharge. The condition was regarded as chronic, progressive polyarthritis of the periarticular type.

Animal Inoculation.—Of three animals injected intravenously with saline suspensions of the cervical discharge, two showed evidence of periarticular hemorrhage and effusion. An admixture of streptococci with a slender gram-negative bacillus was recovered from the joint of one of these animals. Of five rabbits injected with primary cultures three developed purulent arthritis, and cultures from the exudate were positive for a gram-positive diplococcus. Other cultures were negative of growth. Second animal passage with three animals produced joint exudate in one animal. A saline suspension from the joint exudate of this animal was injected intravenously into a rabbit in third animal passage, and a diplococcus in pure culture recovered from the knee joints. Other cultures were negative.

CASE 11 (A361828).—Mrs. A. E. C., aged thirty-seven years, registered at the Clinic in June, 1921. She had been married eight years and had two children, aged five and three. There were no pelvic symptoms except leucorrhea, which had been present for five years, following the birth of her first child. A second pregnancy followed a year later, and shortly afterward she noticed pain in her hands spreading into the joints. The finger joints soon became acutely affected and there followed involvement of the elbow, shoulder, hip, back and knees, in the order named. At the end of four months the condition had progressed so that the patient was unable to walk except with the use of a cane. She had been given foreign protein intramuscularly without benefit, and had received vapor baths, violet ray and massage without in any way influencing the course of the disease.

Examination.—The patient was in a state of fair nutrition. A slight systolic murmur was transmitted to the axilla. There was arthritic involvement of the finger joints, knees, elbows, and wrists, with limitation of motion from pain. Roentgenograms showed hypertrophic arthritis. No focus was discovered. Examination of the pelvis revealed a lacerated cervix, with a mucopurulent cervical discharge.

Animal Inoculation.—Saline suspension of the cervical discharge was injected into one animal which developed arthritic exudate of the knee joints, from which a gram-positive diplococcus was recovered. From this organism a vaccine was made. From one of three rabbits inoculated with primary cultures, the diplococcus was recovered from the joints, kidneys and urine. Of two animals on second passage one developed purulent arthritis. In the third animal passage, but one of five developed joint lesions.

Subsequent Course.—The patient was given a dilatation and curettage, with cautery to the cervical mucous membrane, and hospitalized under general arthritic care. Vaccines were begun in the hospital and continued by her home physician in doses of 10,000,000 raised eventually to 50,000,000 at weekly intervals. A slight general reaction was obtained somewhat irregularly following each administration. The patient has written at intervals over the period of two years reporting a slow but definite improvement. In six months she reported that the pain had largely subsided. Fresh vaccine was supplied at this time and the injections continued until June when she was walking without the aid of a cane. The improvement continued until the winter of 1922, when she contracted several colds, the last of which was associated with a suppurative middle ear infection, following which all her symptoms returned, and when last heard from, in April, 1923, she was confined to bed and suffering an acute exacerbation of all her symptoms.

CASE 12 (A379914).—Mrs. G. M. G., aged twenty-three, registered at the clinic in December, 1921, complaining of "neuritis" of both arms, pain in the shoulder and elbow joints, and stiffness of the back, following the birth of her child in October. Two weeks after the delivery she had had a sudden sharp pain in her shoulder. This was followed by similar pain in the knees, left shoulder and ankles. The process had since involved all of the larger joints.

Examination.—General examination revealed a well-nourished, mentally active young woman. There was some limitation of motion from pain in the right shoulder joint. The search for a focus was unproductive. Examination of the pelvis revealed a patulous cervix with much mucopurulent discharge. The condition was considered beginning polyarthritis of obscure origin. The patient was unable to stay for further investigation. In June, 1922, she returned for an appendectomy on the advice of a physician elsewhere, who had considered the appendix a possible focus. There had been some recent pain and tenderness in the region of the appendix. Operation revealed a catarrhal appendix, 2. Exploration of the gall bladder and pelvis was negative. After the operation, signs of acute involvement of the joints became more apparent, and the patient was hospitalized. At this time both knees were swollen and boggy. In the right knee flexion was limited to 90°, extension to 140°, and there was spasm on extension. Extension of the left knee was 180°, flexion 90°. There was enlargement of the joints of the fingers, with spindle deformity and atrophy. The patient was carefully reexamined for a possible obscure focus and, because of the excessive leucorrhea and the relation of the onset of the condition to the pregnancy, cultures of the cervix and animal inoculation were requested.

Animal Inoculation.—Of five animals injected with saline suspensions of the cervical discharge, two developed lesions of the joints. Eighteen-hour primary cultures from the cervix were injected into seven rabbits. Three developed purulent arthritis, and a diplococcus was recovered in pure culture. Four rabbits were injected on second animal passage, and all developed purulent arthritis. Other cultures, except from the kidneys and urine, were negative.

Bacteriologic Examination of the Deeper Cervical Tissues.—Because the patient continued without improvement under local treatment, with increasing intervals of exacerbation, it was thought advisable to enucleate the infected cervical tissue as a remaining focus. High amputation of the cervix was, therefore, performed and the tissue submitted for culture. The endometrium of the infected cervix was separated as carefully as possible from the deeper structures and cultured separately. The flora obtained was that of the usual cervical discharge. The tissue of the deeper structures, consisting largely of myometrium and terminal glandular tufts, macerated in saline suspension and inoculated into a large number of tubes, was productive of practically unmixed growth of long-chained primarily anaërobic streptococci in twelve of the sixteen tubes inoculated. On blood agar the growth revealed practically pure culture of streptococcus colonies.

This strain obtained from the deeper cervical tissues manifested high virulence for joint tissue, and tested against the leucorrheal strain, produced lesions of the joints in 98 per cent, as against 54 per cent of animals inoculated with the leucorrheal strains of streptococci, and retained its specificity for joints, preserved without subculture in saline-

glycerine suspension for as long as the strain remained viable, a period of more than four months. Four months after isolation all of the four rabbits inoculated with eighteen-hour growths of this suspension developed extensive arthritic involvement.

Serologic Studies.—Inasmuch as febrile exacerbations in the patient gave evidence of repeated autoinoculation, the opportunity was seized of examining the patient's blood during the febrile and postfebrile periods for the presence of antibodies against a possible infecting organism. Following three of such exacerbations, therefore, a series of agglutinations was set up, using the patient's serum and several strains of streptococci, both from the cervix and from other focal sources. The following dilutions of the serum were used: 1:10, 1:50, 1:100, 1:200, 1:300, 1:400 and 1:500. All of the readings were done in duplicate with controls of normal human serum and repeated at daily intervals for six days, during and following each exacerbation. There was no agglutination with the diplococcus obtained from the cervical discharge, or from the more superficial cervical tissues, other than an indefinite flocculation without clearing. Streptococci from other sources were not agglutinated. With the strain obtained from the deeper layers of the cervical tissue, however, definite agglutination with clearing occurred in dilutions of 1:10, 1:50, 1:100, and 1:200 on repeated observation.

In view of this specific relationship between the organism isolated and agglutinins in the patient's serum, it was decided to test the antigenic properties of this strain for animals. A series of rabbits was, therefore, immunized over a period of three months with bi-weekly doses of the organism killed by exposure to heat at 60° for one hour, 1 c.c. of the killed culture in saline suspension, a dose of approximately 8,000,000,000 organisms, being administered. A parallel series of rabbits was similarly treated with a strain of streptococci obtained from a miscellaneous case of cervicitis. Both strains in the fifth week were agglutinated by the homologous serum in dilutions of from 1:6,000 to 1:14,000 with definite clearing. Higher readings were obtained following continued immunization, but not consistently, and spontaneous flocculation often obscured the result. Normal rabbit serum produced no agglutination other than spontaneous flocculation. There was no cross agglutination between the strains. With titration against subculture of the original strain, agglutination became indefinite.

It was believed, on the basis of the apparently high antigenic properties of the strain in rabbits, and the evidence of antibodies in the patient's serum against this streptococcus, that a vaccine of this strain might be utilized in this case therapeutically. Vaccines were, therefore, prepared and given at weekly intervals, in doses of approximately 2,000,000 organisms. General as well as local reaction was marked following each administration, and there was prolonged delay in restitution. Owing to the poor condition of the patient, with periods of frequent autoinoculation, it was thought that continued vaccination might result in too heavy a tax on the natural defences. It was, therefore, discontinued and the patient left the clinic shortly afterward.

REVIEW OF METHODS OF TREATMENT OF ENDOCERVICITIS WITH SUGGESTION OF A NEW METHOD, BASED ON THE PRINCIPLE OF THE GRAM REACTION

The reluctance which clinicians may feel in considering the cervix of the uterus as a possible focus of systemic significance, may be based somewhat on the difficulties widely recognized as attending the complete eradication of a cervical infection. The enucleation of the cervical mucous membrane in the trachelorrhaphy, as done by Emmet or as modified by Dührssen, is insufficient to remove the deeper elements of the infection. A procedure such as the Schroeder operation for the excision of the mucous membrane (modified by Sturmdorf) in its attempt completely to remove the gland-bearing tissues of the cervix, is perhaps the best of the surgical procedures, although whether complete removal of the infected tissue by this means is possible is questionable. Köster, who examined microscopically a

piece of tissue from the new cervical wall, found glandular elements and surrounding inflammatory reaction similar to the tissue removed in the cone-shaped wedge. Amputation of the cervix, as described by Hegar, should remove sufficient tissue to result in eradication of the focus. The continuation of the discharge in some instances, however, following this operation, is evidence that the procedure is not infallible in removing the bacterial infection. The interference with the physiologic functions of the uterus, moreover, following amputation of the cervix, with the tendency to intensify a preexisting menorrhagia or dysmenorrhea, as Köster asserts, renders the

TABLE IV

BACTERIOCIDAL EFFECTS OF ANILINE DYES ON CERVICAL ORGANISMS

DYE	DILUTIONS	STRAINS													
		1		2		3		4		5		6		7	
		5-HOUR	24-HOUR	5-HOUR	24-HOUR	5-HOUR	24-HOUR	5-HOUR	24-HOUR	5-HOUR	24-HOUR	5-HOUR	24-HOUR	5-HOUR	24-HOUR
Gentian violet	1:10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1:50	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1:100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1:500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1:1,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1:5,000	0	0	0	0	0	0	0	0	0	0	+	+	+	0
	1:10,000	+	0	0	0	+	0	0	0	0	0	+	+	+	+
	1:50,000	+	+	+	0	+	+	+	0	+	+	+	+	+	+
Neutral acri-flavine	1:10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1:50	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1:100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1:500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1:1,000	+	+	0	0	0	0	0	0	0	0	0	0	0	0
	1:5,000	+	+	0	0	+	+	0	0	+	+	+	+	+	+
	1:10,000	+	+	+	+	+	+	0	0	+	+	+	+	+	+
	1:50,000	+	+	+	+	+	+	0	0	+	+	+	+	+	+
Mixture	1:10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1:50	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1:100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1:500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1:1,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1:5,000	0	0	0	0	0	0	0	0	0	0	+	+	+	0
	1:10,000	+	0	+	+	+	+	0	0	0	0	+	+	+	+
	1:50,000	+	+	+	+	+	+	0	0	+	+	+	+	+	+

operation unjustifiable except in selected cases. Kennedy, in the presentation of a new method, reviews the general and local measures in current usage, discussing the value of cauterization, tampons, douche powders, paste, curettage, and vaccine as introduced by Curtis, with the conclusion that none of these methods may be relied on to remove the deeper foci of bacteria. However, the method of interstitial injections of ethyl alcohol into the cervical tissue, devised by him because of its bacteriocidal principles and its effectiveness in penetrating uniformly the deeper intersitial tissues with a minimum of damage to the organ itself, has seemed theoretically the most rational procedure in the treatment of chronic and resistant cervical infection. The rationale of this method is based on the effect of alcohol injections

on perineural foci. He observes, "Since the injection of alcohol around the nerve accomplishes, either the chemical section of the nerve, or the obliteration of a perineural focus of bacteria (since the action of the alcohol is quite local), or both, and also since neuralgia has been relieved by the injection of alcohol about the nerve and nerve function has returned without the return of the neuralgia, the alcohol must have obliterated a focus of bacteria. If alcohol used in this way will obliterate a focus of bacteria about a nerve, why will it not obliterate a focus of bacteria interstitially in the cervix and why not a focus of bacteria in any part of the body within the reach of its use?" This method has been used effectively in the Mayo Clinic in a series of cases in which the cervix was edematous, cystic, and characteristic of endocervicitis. The results will be reported by Melson in a separate paper. Owing to the mixed flora present in long-standing cases of cervicitis, and the bacteriostatic properties of the aniline dyes, gentian violet for the gram-positive and neutral acriflavine more specifically for gram-negative bacteria, as developed by Churchman, and in view of a personal communication from Dr. Churchman to the effect that the solution of these dyes is as diffusible as alcohol, if not more so, and as nontoxic, a modification of Kennedy's method was devised, using equal proportions of gentian violet and neutral acriflavine in varying dilutions for interstitial injection. The bacteriostatic effects *in vitro*, summarized in Table IV, reveal an apparent bacteriocidal as well as bacteriostatic effect in dilutions of 1:1,000, with complete suppression of twenty-four hour growth in dextrose-brain-broth. The cervical discharge was examined bacteriologically, with the technic described, before injection, and from the cervical canal following treatment. Several cases studied to date were rendered bacteria-free following a single injection, several showed slight growth in the dextrose-brain-broth tube after the second day of incubation, revealing a survival of a few of the gram-positive staphylococcus and streptococcus forms. A second injection usually rendered the canal sterile. One of the first patients treated, a case of chronic arthritis without other demonstrable focus, experienced prompt relief within several days, and has continued to improve to date, a period of several months. The details of the gentian violet modification of the Kennedy interstitial injection method and its results in a series of cases will be reported later from this clinic.

CONCLUSIONS

1. The anatomic and pathologic facts reviewed point to the structural predisposition of the cervix uteri to act as chronic foci.

2. Bacteriologic and animal experiments indicate the special rôle played by the streptococcus, with regard to pathogenicity, and the relative unimportance of the more saprophytic flora.

3. Evidence is presented to show the affinity of cervical streptococci for joint tissues.

4. Marked percentage increase in joint localization in a series of selected

cases of arthritis, suggests relationship with Rosenow's elective localization theory in focal infection.

5. Clinical and experimental evidence is offered as further support of such a relationship.

6. The antigenic properties of the cervical streptococci suggest low virulence but high specificity of these strains.

7. Evidence is offered to suggest a parallelism between the virulence of the cervical streptococci, and their biologic conditions of growth.

8. Experiments *in vitro* suggest the bacteriocidal, as well as the bacteriostatic effects of the aniline dyes for cervical organisms. A modification of Kennedy's interstitial injection method is offered on the selective bacteriostatic principle for the elimination of the cervical focus.

BIBLIOGRAPHY

[1]Bumpus, H. C., Jr. and Meisser, J. G.: Focal Infection and Selective Localization of Streptococci in Pyelonephritis, Arch. Int. Med., 1921, xxvii, 326-337.

[2]Churchman, J. W.: The Selective Bactericidal Action of Gentian Violet, Jour. Exper. Med., 1912, xvi, 221-247.

[3]Churchman, J. W.: Further Studies on the Behavior of Bacteria toward Gentian Violet, Jour. Exper. Med., 1921, xxxiii, 569-581.

[4]Churchman, J. W.: The Therapeutic Significance of the Gram Reaction, Bull. Johns Hopkins Hosp., 1921, xxxii, 225-227.

[5]Churchman, J. W.: The Mechanism of Bacteriostasis, Jour. Exper. Med., 1923, xxxvii, 543-551.

[6]Curtis, A. H.: On the Etiology and Bacteriology of Leucorrhoea, Surg., Gynec. and Obst., 1914, xviii, 299-306.

[7]Curtis, A. H.: A Combined Bacteriological and Histological Study of the Endometrium in Health and in Disease, Surg., Gynec. and Obst., 1918, xxvi, 178-188.

[8]Curtis, A. H.: Chronic Leukorrhea; Its Pathology and Treatment, Jour. Am. Med. Assn., 1920, lxxiv, 1706-1711.

[9]Curtis, A. H.: Bacteriology and Pathology of Fallopian Tubes Removed at Operation, Surg., Gynec. and Obst., 1921, xxxiii, 621-631.

[10]Döderlein, A.: Das Scheidensekret und seine Bedeutung für das Puerperalfieber, Leipzig, O. Durr, 1892, pp. 86.

[11]Dührssen, A.: Ueber die operative Heilung der mobilen und fixirten Retroflexio uteri auf vaginalem Wege an der Hand von 207 eigenen Operationsfällen, mit besonderer Berücksichtigung der Dauerfolge, Arch. f. Gynaek., 1894, xlvii, 284-448.

[12]Emmet, T. A.: The Principles and Practice of Gynaecology, 3 ed. Philadelphia, Henry P. Lea and Co., 1884, pp. 876.

[13]Hegar, A. and Kaltenbach, R.: Die operative Gynäkologie, mit Einschluss der gynäkologischen Untersuchungslehre, 2 Aufl. Stuttgart, F. Enke, 1881, pp. 773.

[14]af Heurlin, M.: Bakteriologische Untersuchungen des Keimgehaltes im Genitalkanale der fiebernden Wöchnerinnen mit Berücksichtigung der Gesamtmorbidität im Laufe eines Jahres, Helsingfors, 1910, pp. 626.

[15]Hitschmann, F. and Adler, L.: Die Lehre von der Endometritis, Ztschr. f. Geburtsh. u. Gynäk., 1907, lx, 63-86.

[16]Hitschmann, F. and Adler, L.: Der Bau der Uterusschleimhaut des geschlichtsreifen Weibes mit besonderer Berücksichtigung der Menstruation, Monatschr. f. Geburtsh. u. Gynäk., 1908, xxvii, 1-82.

[17]Hitschmann, F. and Adler, L.: Ein weiterer Beitrag zur Kenntnis der normalen und entzündeten Uterusmucosa. Die Klinik der Endometritis mit besonderer Berücksichtigung der unregelmässigen Gebärmutterblutungen, Arch. f. Gynäk., 1913, c, 233-304.

[18]Kennedy, W. T.: Chronic Endocervicitis; A Partial Review of the Literature with an Introductory Paragraph on the Surgical Use of Ethyl Alcohol and a Preliminary Report of the Treatment of Endocervicitis with Ethyl Alcohol Injected Interstitially, Illustrated by Cases, Am. Jour. Obst. and Gynec., 1920-1921, i, 929-940.

[19]Köster, H.: Tracheloplasty for Chronic Endocervicitis, Surg., Gynec. and Obst., 1920, xxxi, 310-311.

[20]Matthews, H. B.: A Study of Chronic Endocervicitis, Surg., Gynec. and Obst., 1921, xxxii, 249-257.

[21]Menge, K.: Bemerkungen zu der Walthard'schen Arbeit. Bacteriologische Untersuchungen des weiblichen Genitalsekretes in Graviditate und im Puerperium, Centralbl. f. Gynäk., 1895, xix, 314-316.
[22]Menge, C. and Krönig, B.: Bakteriologie des weiblichen Genitalkanales, 2 v. Leipzig, Georgi, 1897, 332; pp. 402.
[23]Polak, J. O.: A Manual of Gynecology, 2 ed. Philadelphia, Lea and Febiger, 1922, pp. 396.
[24]Poynton, F. J. and Paine, A.: The Etiology of Rheumatic Fever, Lancet, 1900, ii, 861-869; 932-935.
[25]Rosenow, E. C.: The Etiology of Articular and Muscular Rheumatism, Jour. Am. Med. Assn., 1913, lx, 1223-1224.
[26]Schroeder, C.: Zur Technik der plastischen Operationen am Cervix uteri, Charité-Ann., 1880, v, 343-352.
[27]Stroganoff, V.: On the Bacteriology of the Female Sexual Canal, Monatschr. f. Geburtsh. u. Gynäk., 1895, ii, 365-494.
[28]Strong, L. W.: Physiology and Pathology of the Endometrium, Am. Jour. Obst., 1919, lxxx, 139-150.
[29]Sturmdorf, A.: Gynoplastic Technology, Philadelphia, F. A. Davis Co., 1919, pp. 347.
[30]Walthard, M.: Bakteriologische Untersuchungen des weiblichen Genitalsecretes in Graviditate und im Puerperium. (Ein Beitrag zur rationellen Prophylaxe des Puerperalfiebers), Arch. f. Gynäk., 1894-1895, xlviii, 201-268.
[31]Wegelius, W.: Bakteriologische Untersuchungen der weiblichen Genitalsecrete während der Entbindlichen und des Wochenbettes, mit besonderer Berücksichtingung der Frage von der puerperalen Selbstinfektion, Arch. f. Gynaek., 1909, lxxxviii, 249-390.
[32]Winter, G.: Die Mikroorganismen im Genitalkanal der gesunden Frau, Ztschr. f. Geburtsh. u. Gynäk., 1888, xiv, 443-488.

A COMPARISON OF THE EFFECTS OF THE INJECTION OF GUM ACACIA SOLUTION AND OF THE TRANSFUSION OF BLOOD ON THE OXIDATIVE POWER OF THE BRAIN AS INDICATED BY ALTERATIONS IN TEMPERATURE AFTER THE INJECTION OF ADRENALIN*

By George W. Crile, M.D., Amy F. Rowland, B.S., and S. W. Wallace, M.D.,
Cleveland, Ohio

In the Bio-Physics laboratory of the Cleveland Clinic it has been shown that adrenalin exercises a specific effect on the oxidative power of the brain, which is manifested in normal animals by an increased temperature of the brain in direct relation to the amount of adrenalin injected.[1] The establishment of this specific effect of adrenalin on the brain has provided us with a criterion whereby to test the capacity of certain agencies for increasing or diminishing the oxidative power of the brain—that is, one would expect that if the oxidative power of the brain is increased by the agent which is being tested, then the response of the brain to an injection of adrenalin given within the period of activity of the agent will be increased. Conversely, the response of the brain to the injection of adrenalin will be diminished in the presence of an agent which depresses the oxidative power of the brain.

In view of the variance of opinion regarding the value of gum acacia

*Read before the Physiological Society, Toronto, Canada, December 27-29, 1922.
Received for publication, July 1, 1923.

(Bayliss) solution as a means of conserving the failing energies of the organism after hemorrhage or after shock, and believing that failing powers presuppose among other things a diminished power of work, hence of oxidation within the brain, it seemed desirable to make a comparative study of the effects of gum acacia solution and of transfusion upon the power of the brain to respond to adrenalin. A six per cent gum acacia solution was used, prepared according to the formula of Bayliss[2], the solution being used at a temperature of 38.75° C.

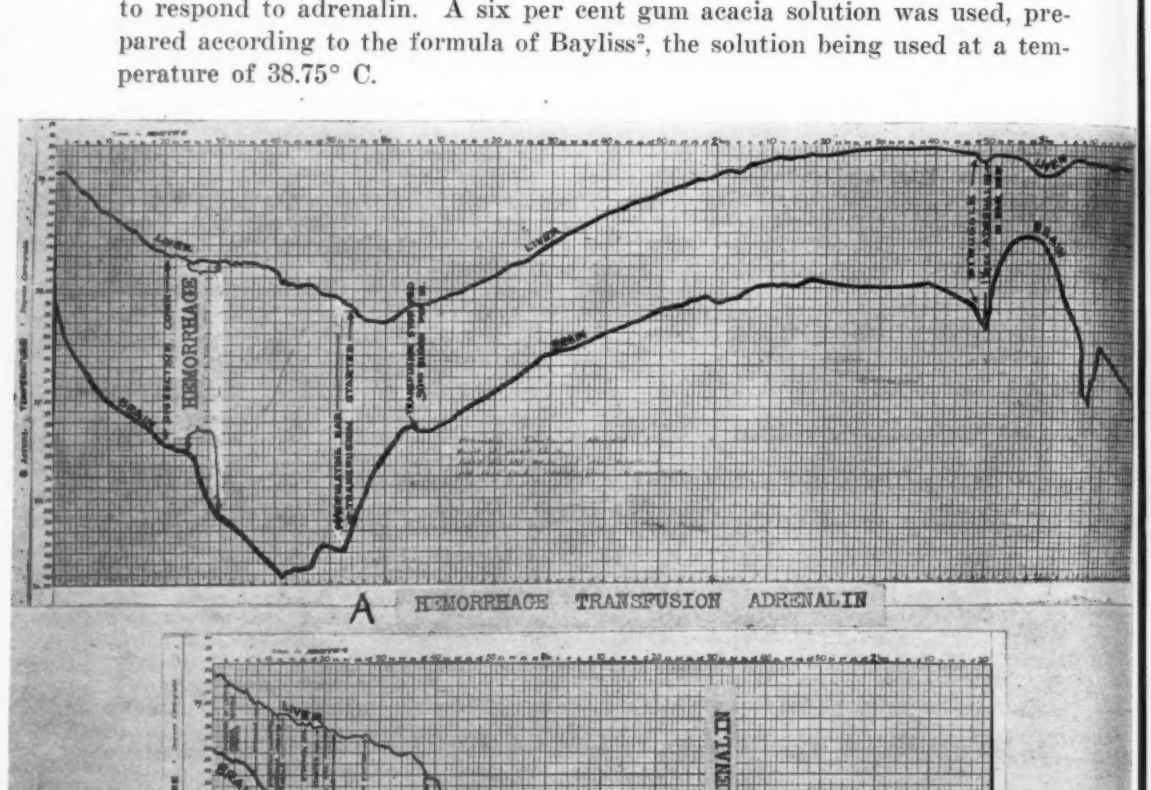

Chart 1.—Temperature charts showing effect upon the temperature of the brain and the liver of (A) adrenalin injection after transfusion, and (B) adrenalin injection after the infusion of Bayliss solution.

These experiments were performed on healthy Belgian hares of from five to seven pounds. From 30 to 50 c.c. of blood was withdrawn from each, the quantity in each case being determined by the clinical condition of the animal. None was reduced to extreme prostration. The temperature variations in the brain and the liver were measured by especially constructed thermocouples.

Note 2.—In experiment 3 the temperature fell 0.6° C. during the infusion and continued to fall for a period thereafter—making the rise only 1½ minutes.
Note 3.—Thermocouple broke while temperature of brain was still rising after adrenalin injection.

TABLE I

COMPARISON OF THE EFFECTS OF GUM ACACIA (BAYLISS) SOLUTION AND OF TRANSFUSION OF BLOOD ON THE RESPONSE OF THE BRAIN TO ADRENALIN

I *Gum Acacia*

EXPER. NO.	WT. OF RABBIT	AMOUNT OF HEMORRHAGE	AMOUNT OF INFUSION OR TRANSFUSION	FALL IN T. OF BRAIN DUE TO HEMORRHAGE	TOTAL RISE IN T. OF BRAIN AFTER INFUSION OR TRANSFUSION	PERIOD OF RISE	RISE AFTER ADRENALIN	PERIOD OF SUSTAINED RISE AFTER ADRENALIN	MAX. T. AFTER ADRENALIN AS COMPARED WITH T. BEFORE HEMORRHAGE	MAX. T. AFTER ADRENALIN AS COMPARED WITH MAX. T. AFTER INFUSION OR TRANSFUSION	PERIOD OF SUSTAINED MAXIMUM RISE	
1	6 lbs.	35 c.c.	40 c.c.	−0.68° C.	+0.52° C.	7½ Min.	0.16° C.	2 Min.	−0.17° C.	−0.05° C.	30 Sec.	
2	5 lbs.	30 c.c.	35 c.c.	−0.32° C.	+0.68° C.	14 Min.	0.45° C. (See Note 1)	0 Min.	+0.04° C.	−0.31° C.	0 Min.	
3	5½ lbs.	35 c.c.	45 c.c.	−0.5 ° C.	Fall −0.21° C.	(net) 1½ Min. (See Note 2)	0.25° C.	0 Min.	−0.84° C.	−0.09° C.	30 Sec.	
4	4½ lbs.	25 c.c.	35 c.c.	−0.7 ° C.	Fall −0.8° C.	10 Min.	0.1° C.	0 Min.	−0.45° C.	−0.17° C.	0 Min.	

II *Transfusion*

5	7 lbs.	50 c.c.	60 c.c.	0.94° C.	2.66° C.	3 Hrs., 52 Min.	0.28° C.	0 Min.	+1.12° C.	+0.25° C.	9 Min.	Still sustained when adrenalin was given.
6	6⅓ lbs.	40 c.c.	45 c.c.	0.48° C.	1.09° C.	2 Hrs., 1 Min.	Dose I 0.73° C. Dose II 0.45° C.	Dose I 1 Min. Dose II 10 Min.	Dose I +1.09° C. Dose II −0.49° C.	Dose I +0.13° C. Dose II +0.4° C.	0 Min.	
7	6 lbs.	35 c.c.	40 c.c.	0.41° C.	1.83° C.	3 Hrs., 37 Min.	0.17° C.	11 Min.	+1.41° C.	+0.11° C.	12 Min.	Still sustained when adrenalin was given.
8	6½ lbs.	40 c.c.	50 c.c.	1.15° C.	2.18° C.	1 Hr., 23 Min.	0.84° C.	3 Min.	+1.94° C.	+0.39° C.	2 Min.	
9	5 lbs.	35 c.c.	40 c.c.	0.9° C.	1.65° C.	1 Hr., 16 Min	0.25° C.	(See Note 3)			25 Min.	Still sustained when adrenalin was given.

Note 1.—In experiment 2 the adrenalin was given 55 minutes after the maximum rise in temperature when the temperature had fallen 0.76° below the maximum. In all other experiments the adrenalin was injected just as the temperature began to fall after the maximum had been reached.

Note 2.—In experiment 3 the temperature fell 0.6° C. during the infusion and continued to fall for a period thereafter—making the *net* period of rise only 1½ minutes.

Note 3.—Thermocouple broke while temperature of brain was still rising after adrenalin injection.

When the maximum effect of the hemorrhage had been produced, as registered by the cessation of the fall in temperature of the brain, four of the animals were given an intravenous infusion of the gum acacia solution, the remaining four receiving a transfusion of citrated blood taken from another rabbit. Table I shows the variations in temperature in each of these animals. The significant columns are those which show the maximum temperature attained after the injection of adrenalin as compared (a) with the temperature before the hemorrhage and (b) with the maximum temperature attained after the infusion or transfusion. In every animal which received the transfusion of blood the temperature after the injection of adrenalin rose to a level of from one to nearly two degrees above the temperature of the animal before it was subjected to the hemorrhage. On the other hand, in only one of the three which received the gum acacia solution did the temperature after the adrenalin injection rise even to its level before the hemorrhage. In no instance after the gum acacia infusion was the adrenalin able to raise the temperature of the brain to the maximum attained after the infusion, while in every animal which received the transfusion the maximum rise after the transfusion was surpassed after the injection of adrenalin.

Another significant feature is the length of time after the infusion or transfusion of blood was given, during which the temperature of the brain continued to rise. The longest period of rise after the gum acacia infusion was 14 minutes. On the other hand, the *shortest* period of rise after the transfusion of blood was 1 hour and 23 minutes, the *longest* 3 hours and 52 minutes. The clinical symptoms of the animals throughout corresponded to the temperature observations. In all four of the animals subjected to the gum acacia solution the temperature of the brain fell to a very low level before the termination of the experiment, and the animals were practically *in extremis* (Chart I-B). Of the four animals subjected to blood transfusion, all were in good condition at the end of the experiment, and the temperature of each was but little below that at the beginning of the experiment (Chart I-A).

CONCLUSION

A comparison of the response of the brain to the injection of gum acacia solution and to the transfusion of whole blood indicates that the oxidative power of the brain is markedly diminished by the gum acacia solution, while it is increased by the transfusion of whole blood.

REFERENCES

[1]Am. Jour. Physiol., 1922, lxii, 370-382; Proc. Am. Phil. Soc., 1922, lxi, 237-245.
[2]Bayliss, W. M.: The Medical Bulletin, Paris, 1918, No. 6, p. 462.

A PROPOSED BUFFERED PHYSIOLOGIC SALT SOLUTION TO BE USED IN SEROLOGY*

EDWARD C. MASON, M.D., AND ARTHUR H. SANFORD, M.D., ROCHESTER, MINN.

WITH the introduction of the standardized Kolmer technic we have had a great deal of trouble with the amboceptor titration. Apparently the more care we have exercised in selecting reagents, such as double or triple distilled water and chemically pure sodium chloride, the less satisfactory have been our results. Early in the use of the Kolmer technic we were of the opinion that the most likely source of error came through the salt solution. Of the various salt solutions used those made up in tap water or spring water were generally satisfactory in yielding constant results, while those made up in carefully distilled water with chemically pure sodium chloride, were almost invariably unsatisfactory.

About this same time while making physicochemical determinations on amboceptor, complement, and erythrocytes as components of a hemolytic system, it was observed that salt solutions prepared with double distilled water were usually not neutral but acid. It was also noted that determination of the acid concentration at the onset of a test and one hour later might show an entirely different acid concentration. Such salt solutions are too sensitive to permit the use of phenyl red as an indicator in determining their hydrogen-ion concentration.

PROCEDURE

If a few drops of phenyl red are added to 10 c.c. of physiologic salt solution, prepared with double distilled water, a color characteristic of a solution with a hydrogen-ion concentration slightly below the neutral point will generally be obtained. If the tube is shaken, the color will fade rapidly, and should the breath be blown across the tube, the color will leave instantly; the resulting salt solution is then acid beyond the range of phenyl red. However, if the same solution is boiled, the red of phenyl red will again return, and will generally be of an intensity characteristic of a higher hydrogen-ion concentration than the original salt solution. If the foregoing experiment is repeated, but using tap water instead of double distilled water, it will be noted that shaking or breathing across the solution is not accompanied by such a rapid color change, and also that the color is never reduced to that of the double distilled water.

With this simple experiment in mind we have carried out a series of experiments to determine the possible changes to be produced by introducing

*From the Section on Medicine and Clinical Pathology, Mayo Clinic, Rochester, Minn.
Received for publication, September 3, 1923.

carbon dioxide into physiologic salt solution, and by boiling such a solution. Three samples of water were chosen, double distilled water, tap water, and spring water. All titrations were carried out on the same cells, amboceptor and complement; and the same salt solution was used throughout the test. Nine combinations were tested the same morning. Table I is an outline of the results obtained with salt solutions prepared with the three types of water without treatment. The double distilled salt solution became slightly acid during the test, and also gave a poor titration (1:2000); the other two solutions became slightly more alkaline, and gave a good titration of 1:6000.

The results recorded in Table II were obtained with the solutions used in Table I, except that all were boiled for ten minutes and cooled without contact with air containing carbon dioxide. The boiling markedly increased the hydrogen-ion concentration of all three solutions, and again the double distilled solution became more acid during the test. Apparently the other two solutions became too alkaline for the optimal titration.

TABLE I

UNTREATED SALT SOLUTIONS

WATER	BEFORE TEST pH	AFTER TEST pH	AMBOCEPTOR TITRATION
Double distilled	7.0	6.4	1:2000
Tap	7.8	8.2	1:6000
Spring	6.8	7.0	1:6000

TABLE II

BOILED SALT SOLUTIONS

WATER	BEFORE TEST pH	AFTER TEST pH	AMBOCEPTOR TITRATION
Double distilled	7.8	7.0	Nil
Tap	8.4	8.4	1:30000
Spring	9.4	9.4	Nil

TABLE III

SOLUTIONS TREATED WITH CARBON DIOXIDE

WATER	BEFORE TEST pH	AFTER TEST pH	AMBOCEPTOR TITRATION
Double distilled	4.4	4.4	Too acid
Tap	5.8	5.8	1:3000
Spring	4.6	4.6	Too acid

Table III shows the results obtained by passing carbon dioxide through the original solution for ten minutes. Owing to the high acidity of the double distilled and spring water, it was impossible to use them for washing cells. The tap water, although becoming quite acid, still gave a titration of 1:3000.

From these data it will be observed that tap water was the least sensitive to boiling and to the addition of carbon dioxide, while the double distilled water was the most sensitive to such agents. These results are due to the fact that the tap water carries a natural buffer, while the double distilled water does not.

PREPARATION OF THE BUFFERED PHYSIOLOGIC SALT SOLUTION

Following a series of observations it was decided that the best titrations were obtained with salt solutions not more acid than pH 7.0 nor more alkaline than pH 8.4. It was also observed that the salt concentration was capable of completely changing the titration. The first buffered salt solution tried was prepared without sodium chloride, and contained only the phosphates in the proportion given by Clark. Such a solution gave a good titration (1:8000) with cells washed in the laboratory salt solution; however, being hypotonic to physiologic salt solution it was thought advisable to make it isotonic by the addition of 3 gm. of sodium chloride for each liter. This mixture gave a poor titration of 1:3000. This experiment influenced us to avoid mixed salt solutions and we attempted to prepare a phosphate buffered solution of sufficient concentration to be also isotonic. Such a solution may be prepared by using any proportion of the two phosphates in concentration of 0.1 gram molecular weight for each liter. Such a solution with a pH of 8.0 was tried and gave an exceedingly poor titration.

Finding the excessive amounts of the phosphates so unsatisfactory, we next tried the smallest amount of alkaline phosphate that would be sufficient to buffer any acid constituents which were likely to be present during a normal titration. The following proportions were decided upon: 6.8 gm. of sodium chloride, and 1.7 gm. of secondary sodium phosphate for each liter of double distilled water. However, with carefully distilled water this gave a hydrogen-ion concentration of 9.0 and a very poor titration of 1:2000. The modification as follows was tried and found satisfactory: sodium chloride 7.0 gm., secondary sodium phosphate (2 molecules of water) 1.7 gm., and primary potassium phosphate 0.2 gm. for each liter of double distilled water; such a solution will have a hydrogen-ion concentration of 7.4 to 7.8. After using this solution for several months we feel justified in offering it as a physiologic salt solution of definite and constant composition. Other buffers such as calcium hydroxide and various carbonates have been tried, but none has been as satisfactory as the phosphate buffers.

DISCUSSION

In attempting to standardize serologic technic, and thereby place it on a scientific basis, it seems very important that we consider the effect of hydrogen-ion concentration, for it can be definitely demonstrated that variations in the salt or acid concentration are capable of completely reversing results. Therefore, a constant physiologic salt solution which will permit universal comparison of standards is urgently needed. A search of the literature has not revealed that this point has been considered in serology. Williams and Madeleine Swett have made some very interesting observations in studying the hydrogen-ion concentration of solutions for intravenous injections. This work did not influence ours at the time, and is mentioned now because of the corroborative evidence. The observations made by these authors regarding the pH of distilled water, and tap water, are very simil- to ours, and their conclusion with regard to the necessity of using a buffered

salt solution of pH 7.4 for intravenous solution is most suggestive when compared with our experimental optimum pH of solutions for serology. We have not used their buffered solution, as for our purpose our proposed solution seems to be more easily prepared.

CONCLUSIONS

1. There is a close relationship between serologic reactions, hydrogen-ion concentration, and salt concentration, the optimal hydrogen-ion concentration for the amboceptor titration being in the zone of pH 7.0 to pH 8.4.

2. There is need for a physiologic salt solution having a definite and constant composition, both as to salt concentration and hydrogen-ion concentration. Such a salt solution would permit universal comparison of standards.

3. It is impossible to prepare a standard salt solution using tap water, due to the fact that the composition of tap water varies markedly with location. It is equally impossible to prepare a solution with a definite hydrogen-ion concentration from unbuffered double distilled water.

4. We propose a buffered physiologic salt solution having a definite and constant composition, the formula being 7.0 gm. of sodium chloride 1.7 gm. of secondary sodium phosphate (2 molecules of water) and 0.2 gm. of primary potassium phosphate for each liter of double distilled water. This combination yields a solution with pH of 7.4 to 7.8 and a salt concentration of 8.9 gm. for each liter. Such a solution has been giving uniform and constant results.

REFERENCES

Clark, W. M.: The Determination of Hydrogen Ions. 2 ed. Baltimore, Williams and Wilkins, 1922, p. 107.

Williams, J. R. and Swett, Madeleine: Hydrogen Ion Concentration Studies on Distilled Water, Physiologic Sodium Chlorid, Glucose, and Other Solutions Used for Intravenous Medication. Jour. Am. Med. Assn., 1922, lxxviii, 1024-1026.

HEMOLYTIC STREPTOCOCCUS OF HUMAN ORIGIN IN ITS RELATIONSHIP TO HEATING AND FREEZING IN THE MILK INDUSTRY*

By T. D. Beckwith, Ph.D., and B. U. Eddie, M.A., Berkeley, Calif.

It is a a matter of grave importance that milk consumed by human beings be rendered safe before use. Ordinarily this is done by the process of pasteurization, a term which is very broadly interpreted since there is great variation not only with regard to the time of treatment but also as relates to the temperature of exposure. The aim, of course, is to cause the death of any pathogenic organisms which possibly may be present in the raw product. It is well known that pasteurization does not imply complete sterilization,

*From the Department of Bacteriology and Experimental Pathology, University of California.

Received for publication, July 10, 1923.

since Ayers and Johnson[1] have proved that there are many organisms which resist the customary exposure to heat and that some of these forms occur as chains. Considerable work has been done showing that the thermal death point of pathogenic bacteria lies below the ordinarily used temperatures in pasteurization. Seemingly the hemolytic streptococcus, however, has been given little attention in this regard. It was determined, therefore, to study both the effects of heating and of freezing upon this microorganism.

The significance of Streptococcus hemolyticus in milk is well recognized. Particularly has this been the case since the period of the Boston epidemic of "septic sore throat" in 1911 with its 1034 cases and 38 deaths. For a considerable period it was the belief of many authors that the origin of the streptococci was to be found in the animal, but the work of Krumwiede and Valentine[2] and of Davis and Capps[3] indicates that the animal is the secondary host, having been infected originally by a human carrier. An abrasion of the teat is a possible portal of entry. Infection back to other human beings then takes place readily when appropriate conditions present themselves.

The strain of hemolytic streptococcus used in the following experiments was derived in the first place by cultivation from a fatal case in the state of Texas during the war period, and is the one which has been utilized by members of this department in a considerable series of publications in recent years. It has been denominated in our records as "Strain H." Propagation has been followed both by cultivation *in vitro* on blood agar and by constant passage *in vivo* in rabbits by artificially produced empyema.

The first series of experiments was devoted to an endeavor to learn somewhat precisely the effect of heat upon this strain and was carried out as follows:

The period of lag in milk when heated in a test tube in a water-bath was first determined. Fifteen c.c. of fresh skim milk were placed in a test tube suspended in the bath. Two thermometers previously tested for correctness were used, one having its bulb immersed in the milk, while the other was placed to the same depth in the bath close by the tube. Observations were then made to learn how long a time elapsed before the two readings were the same, when the cold tube containing the milk was placed in the bath already brought to the temperature desired. With the bath temperature at 50°, this lag was 3 minutes, while at 75° it was 9 minutes, and with appropriate variations at temperatures intervening. These figures thus obtained were used in all future experiments.

Our first attempt was to learn the effect of heat upon this hemolytic organism in milk as shown by direct streaking from time to time during the period of treatment upon blood agar. This particular tube was from that portion of the strain which had not been in the animal for many generations. Into 15 c.c. of the fresh and sterile skim milk was placed 0.1 c.c. of a 24 hour glucose broth culture which had been incubated at 37° C. The tube thus prepared after careful shaking was placed immediately in the water-bath already heated to the temperature desired. A separate tube was used for every temperature tested and the same tube was used throughout each tem-

perature. At the times indicated, a streak was made upon a rabbit blood agar plate by transfer, using a 3 mm. platinum loop. This plate was then incubated for 48 hours and results noted. Colonies were verified by the zone of hemolysis together with a confirmatory gram stain. Results are shown in Table I. Plus signs show the relative number of colonies, while no growth, and therefore sterilization, is indicated by a zero.

TABLE I.

THE EFFECT OF HEAT UPON THE HEMOLYTIC STREPTOCOCCUS IN MILK. INOCULUM FROM THE MILK IS 1-3 MM. LOOP. ORGANISMS CULTIVATED IN VITRO

TEMPERATURE	IMMEDIATE	10 MIN.	15 MIN.	20 MIN.	25 MIN.	30 MIN.
50	+++	+++	+++	+++	+++	+++
55	+++	+++	+++	+++	+++	++
60	+++	++	++	++	++	+
65	+++	+	+	+	+	+
70	+++	0	0	0	0	0

As has been noted previously, the strain of streptococcus has been cultivated by continuous passage through intrapleural injection in rabbits as well as in the test tube over a period of years. In the former procedure the pleural exudate, when pipetted off at necropsy, is found, as has been noted by Gay and Rhodes[4] to be a pure culture and when placed in a sterile tube in the ice box retains life and virulence for some weeks. The succeeding passage is made in turn from such material. No attempts have been made to determine the differences in pathogenic powers of these two portions of the original strain but it seems fair to assume that virulence is probably fully as great in that which has been carried in the animal body as in that which has been transferred in test tube culture.

TABLE II

THE EFFECT OF HEAT UPON HEMOLYTIC STREPTOCOCCI IN MILK
INOCULUM FROM MILK 1-3 MM. LOOP. ORGANISMS CULTIVATED IN VIVO

TEMPERATURE	IMMEDIATE	10 MIN.	15 MIN.	20 MIN.	25 MIN.	30 MIN.
55	+++	+++	+++	+++	+++	+
60	+++	++	+	+	0	0
65	+++	+	0	0	0	0
70	+++	0	0	0	0	0

With the above facts in mind we next endeavored to determine whether there is any difference to be noted when organisms cultured *in vivo* are used rather than those held continuously *in vitro*. Technic was followed identical with that the results of which are shown in Table I, with the only difference that the inoculum was pleural exudate rather than glucose broth culture. The exudate had been removed from the animal only a few hours when used, and was proved subsequently to contain a high count of the form. The preliminary trial indicated that such organisms are more susceptible to heat than when carried in culture, and to check this outcome, the series of experiments were carried to conclusion three times with results which are set forth in Table II.

It will be observed that the amount of milk which may be removed by

means of a loop is exceedingly small. The thought arises, therefore, that possibly results which are noted in the preceding paragraphs are not final, but that on the other hand this streptococcus is even more resistant to heat than indicated. This would appear to be extremely likely since it is conceivable that the number remaining in the tube may be so small that the loop might not have picked out any of those surviving the treatment. With this matter in mind, a partial check was included, by placing the tubes in the incubator after heating, and there they remained for 24 hours in order to allow multiplication of any possible surviving remainder to take place in the original 15 c.c. portions of the milk. They were then streaked out on blood agar plates. Results obtained through such procedure were identical with those noted in Tables I and II. We are thus forced to the conclusion that Streptococcus hemolyticus of human origin, when present in milk, is comparatively resistant to the effects of heat. However, the effectiveness of heat administration is greater when the organism has been retained in its natural environment than when placed under artificial cultivation.

The efficiency of pasteurization not only is of vital importance to the dairy industry, but it pertains most closely to the manufacture of ice cream inasmuch as the "mix" before freezing is again heated in order to increase the assurance of obtaining a final product both of good grade and of satisfactory keeping quality. It should be stated that it is customary to prepare a standard mixture comprising the milk, sugar, gelatine and other substances, less the flavoring material, in this industry and that such solution is termed the "mix." To this in turn is added the flavoring material, as occasion demands, just preliminary to the freezing process and following the application of heat. The question of possible survival of the hemolytic streptococcus thus becomes intimately related to the preparation of ice cream.

Our next step, therefore, was to determine the effect of pasteurization at different temperatures upon the organism in question when placed in the milk-sucrose-gelatine solution above described. The experiments outlined in the preceding portion of this article were then repeated, and with technic identical except for the substitution of the mixture for skim milk. Results were closely parallel, for it was shown that with organisms cultured in the test tube, sterilization was not affected by the application of 65° heat for 20 minutes, although there was an entire disappearance of the streptococcus at 70° for 10 minutes. Again it was demonstrated that organisms previously cultivated *in vivo* are more susceptible since they resisted 60° for 20 minutes, although killed by 65° for 10 minutes.

Inasmuch as it has been proved that the Streptococcus hemolyticus is not easily killed by heat either in milk or in ice cream mix, it becomes advisable to learn whether the subsequent application of freezing temperatures used during the preparation and storage of the product may be effective to rid the material of the organism. This we were enabled to do through the courtesy of the Dryden Corporation of Oakland, California who were so kind

as to place at our disposal facilities for carrying through the needed experiments.

From the publications of Pennington[5] and her collaborators, together with others who have dealt with the bacteriology of cold stored materials, it would appear to be likely that mere freezing may be expected to be without influence upon the viability of the streptococcus, inasmuch as it has been shown that not only does cold temperature not kill certain similar organisms, but that on the other hand they even multiply somewhat rapidly under such conditions. Furthermore, Smith and Swingle[6] early proved that the temperature of liquid air is ineffective against a series of bacterial forms even when the exposure extends over a considerable period of hours.

In order to determine whether continued freezing kills the hemolytic streptococcus in the stock mix of ice cream, small quantities of this fluid were placed in Florence flasks of 200 c.c. capacity. The level was such that it was slightly below the point of greatest diameter so that expansion accompanying freezing might not cause the container to burst. These were then autoclaved for 15 minutes at 15 pounds' pressure. When cool 0.1 c.c. of a 24 hour glucose broth culture was inoculated into each of these flasks which were then placed in the hardening room of the Dryden Corporation. The temperature maintained in this room closely approximates –20° C. continuously. On each succeeding day, one of the flasks thus prepared was removed and its contents streaked on to rabbit blood agar, in order to determine the presence of the hemolyzing organism. It was found that death takes place under these conditions in one week's time.

Inasmuch as the period of survival of Streptococcus hemolyticus in frozen ice cream mix is so short, one is compelled to consider whether it is the effect of the degree of cold or whether possibly this is not caused by osmosis due to the large amount of sucrose (15 per cent) present in the liquid. To differentiate between these two possibilities, there was placed in Florence flasks, and with the same precautions as have been outlined in the preceding paragraph, some fresh skim milk to which had been added 15 per cent of cane sugar. After autoclaving for 15 minutes at 15 pounds' pressure and then cooling, each of these flasks was inoculated with 0.1 c.c. of a 24 hour glucose broth culture. One of these preparations was then retained at room temperature varying between 17 and 22° while the rest were frozen at –20° C. Tests made by streaking on to blood agar at frequent intervals showed that the organisms died out of the frozen material in 20 days and that they disappeared from the check flask retained unfrozen in 17 days. This evidence that the effect is due to the presence of the sugar rather than to the influence of the low temperature is strengthened by the fact that it was indicated by means of a parallel experiment that sterilization takes place in ice cream mix held at room temperature within 17 days. Moreover, when skim milk is prepared in flasks as above outlined, inoculated and then held at the low temperature used, there is no apparent diminution of the organisms present within a period of six weeks.

The results herewith presented have a certain degree of importance to

the problem of public health in its relationship to the pasteurization of milk. It is shown that hemolytic streptococcus of human origin may resist the temperatures and times of application ordinarily specified in the legislation of some of our states and provinces. It must be admitted that from epidemiologic evidence available, it appears that the ordinary formulas for the treatment of raw milk are satisfactory. That is, pasteurization stops further spread through milk of an infection produced by this organism. However, in the light of the experimental work described above, it seems evident that this does not imply necessarily the death of the infecting agent. It does seem probable, however, that treatment by means of heat either reduces the numbers of organisms in point of concentration below the minimum infecting dose, or that even if they are not killed, the virulence of the surviving remainder is reduced. No attention thus far has been given to either one of these points.

RESULTS

Hemolytic streptococcus of human origin in milk is relatively resistant to pasteurization. The same is true when it occurs in ice cream stock mixture.

The organism when grown in a lesion for purposes of multiplication is slightly more susceptible to the effects of heat than when cultivated repeatedly in the test tube.

Should this streptococcus survive pasteurization in the stock syrup used in the ice cream industry, it nevertheless is killed fairly readily by the high sugar content.

It is uninfluenced by freezing temperature at –20° C.

REFERENCES

[1]Ayers and Johnson: Bureau of Animal Industry, 1913, Bull. 161.
[2]Krumwiede and Valentine: Coll. Stud. Bureau of Laboratories, City of N. Y., 1914-15, viii, 171.
[3]Davis and Capps: Jour. Inf. Dis., 1914, xv, 135.
[4]Gay and Rhodes: Jour. Inf. Dis., 1922, xxxi, 101.
[5]Pennington and Walker: N. Y. Med. Jour., 1907, lxxxvi, 1013.
[6]Smith and Swingle: Science, 1905, n.s., xxi, 481.

EFFECTS OF THE INTRAVENOUS INJECTIONS OF ACRIFLAVINE*

By Harvey Spencer, New Haven, Conn.

Of the various new antiseptics which have been studied during the past few years probably none have received greater attention than the flavine compounds. Acriflavine in particular has been advocated not only as a surface disinfectant, but also as an internal antiseptic, and although the literature contains many references to the several uses of the dyestuff, relatively little work has been reported showing the mode of action of the compound and the incidental effects which the dye may exert in addition to its bactericidal action.

Of primary importance is the fact, originally noted by Browning, Kennaway, Gulbransen and Thornton,[7] that acriflavine does not diminish in effectiveness by contact with organic fluids. This conclusion has been confirmed, in part at least, by Davis,[10] Fleming,[16] and Drummond and McNee,[14] and has served as a basis for the utilization of the compound by intravenous injection in septicemic conditions. Bohland[2] reports on the therapeutic use of acriflavine in influenza, pneumonia, sepsis, pyelonephritis, and Bacillus coli pyelitis. As a corollary to these observations it has been claimed that the dye exerts other actions which render of questionable expediency its introduction into the circulation. Thus, Bohland states that it acts as an erythrocytic agglutinant. Fleming,[15] Morgan,[19] and Browning and others,[7] have stated that the dye brings about the destruction of leucocytes while Davis[10] affirms that phagocytosis is still active in concentrations of the dye which are two hundred times the concentration which is bactericidal. As to the action of acriflavine on serum, the observations are conflicting. Fleming[15] states that the dye is an anticoagulant, whereas Browning and Ligat[8] state that the dye favors coagulation. In its practical application, aside from its use as a wound antiseptic and an agent of intravenous medication, the dye has been most widely applied as a urinary antiseptic, as has been reported by Davis.[10] For these several uses various concentrations of the dyestuff have been used. For intravenous use the compound has been dissolved in physiologic saline and injected in such amounts as would give a concentration of 1:20,000 for the entire blood volume.

From this cursory review of the literature it is clear that contradictory results have been reported as to many of the incidental properties of the dyestuff and the following experimental data bear upon certain of these properties, such as the effect of acriflavine upon red and white blood cells, and the bactericidal action to be expected from the intravenous injection of the compound.

*From Brady Laboratory of Pathology and Bacteriology, Yale University School of Medicine, New Haven, Conn.

Received for publication June 12, 1923.

ACTION OF ACRIFLAVINE UPON ERYTHROCYTES

(a) Agglutinating Action.—In determining the agglutinating action of acriflavine all solutions of the dye and all suspensions of the cells were made in physiologic saline solution. Human erythrocytes, unwashed, were mixed upon microscopic slides with acriflavine to yield dilutions of the dyestuff of 1:2,000, 1:4,000, 1:8,000, progressing to 1:512,000. Cover glasses were applied and the edges sealed to prevent evaporation. During the period of examination, 75 minutes, no agglutination could be detected. In the highest concentration, that is 1:2,000, evidence of hemolysis was noted, the indistinct forms of the red cells first appearing between ten and fifteen minutes after the cells were mixed with the dye. The results of this experiment fail to confirm the report of Fleming, inasmuch as in these concentrations, at least, no agglutination was observed. The suggested hemolytic action agrees with the statement of Bohland previously cited and further tests were conducted to more accurately determine this factor.

(b) Hemolytic Action.—The capacity of the dyestuff to bring about hemolysis of red cells was determined both *in vitro* and *in vivo*. The test tube experiments were performed with human erythrocytes, the results being checked both macroscopically and microscopically. *In vivo* experiments were conducted in rabbits by injecting the dyestuff intravenously and making red blood cell counts at intervals following such injections. Table I indicates the effect of acriflavine, in the concentrations indicated, upon human red blood cells, thoroughly washed and prepared in a 5 per cent suspension in physiologic salt solution. As indicated, two readings were taken, one after 24 hours in the ice box and the other after an additional week at room temperature.

TABLE I

ACRIFLAVINE DILUTION	HEMOLYSIS AFTER 24 HOURS	HEMOLYSIS AFTER ONE WEEK
Control	No hemolysis	Total hemolysis
1:1,000	Total hemolysis	Total hemolysis
1:2,000	Total hemolysis	Total hemolysis
1:4,000	Partial hemolysis	50% hemolysis
1:8,000	No hemolysis	No hemolysis
1:16,000	No hemolysis	No hemolysis
1:32,000	No hemolysis	No hemolysis
1:64,000	No hemolysis	No hemolysis
1:128,000	No hemolysis	No hemolysis
1:256,000	No hemolysis	No hemolysis

It is obvious from Table I that acriflavine in concentrations as high as 1:4,000 bring about a considerable degree of hemolysis, although in the higher dilutions it appears that the dyestuff is protective, since after the lapse of one week all of the tubes containing small amounts of the dye fail to show hemolysis, while the control tube, unprotected against bacterial contamination and growth, is completely hemolyzed. Microscopic determination of the effect of the dye upon red blood cells showed that after contact for 24 hours slight hemolysis was to be found with the dyestuff in a concentration of 1:8,000, while in higher dilutions hemolysis did not appear. One further *in vitro* test of the hemolytic action of acriflavine was made,

using the dyestuff in its critical dilution, 1:4,000. These tests were conducted by mixing washed cells and the dyestuff in hemocytometer pipettes. Counts were made immediately after mixing and again after 24 hours, the tests being performed in triplicate. Table II shows the counts secured in one such series of determinations.

TABLE II

CONTROL RED BLOOD CELL COUNT	COUNT AFTER 24 HOURS	PER CENT OF HEMOLYSIS
(a) 5,912,000	4,524,000	24%
(b) 4,752,000	2,360,000	51%
(c) 6,248,000	3,448,000	45%

The ability of acriflavine to produce hemolysis *in vivo* was determined by injecting the dyestuff intravenously into rabbits to a concentration of 0.1 of a gram per kilogram of body weight. Blood counts, both red and white, were made prior to the injection of the dye and subsequent to the injection at intervals of one hour, three hours, and twenty-four hours. In Table III the red blood cell counts are given for four rabbits treated in this way, and in two instances the leucocyte counts are also given. These counts demonstrate that within one hour after the injection an hemolysis occurs amounting to approximately 500,000 red blood cells per cubic millimeter. The extent of the hemolysis did not increase appreciably during the next two hours, and at the end of twenty-four hours the cell counts had returned closely to normal. The action of the dyestuff upon the leucocytes was more pronounced. Within the three-hour period a leucocytosis of over 100 per cent developed and this was only somewhat reduced by the end of twenty-four hours. The results reported above do not indicate that there is a marked leucocytic destruction as the result of the injection of the dyestuff.

TABLE III

	RABBIT NO. 1		RABBIT NO. 2	
	R. B. C.	W. B. C.	R. B. C.	W. B. C.
Control count	6,300,000		5,900,000	
1 hour after injection	5,568,000		5,146,000	
3 hours after injection	5,752,000		5,120,000	
24 hours after injection	6,124,000		5,476,000	
	RABBIT NO. 3		RABBIT NO. 4	
	R. B. C.	W. B. C.	R. B. C.	W. B. C.
Control count	6,425,000	8,700	5,704,000	8,800
1 hour after injection	6,556,000	10,800	5,456,000	9,200
3 hours after injection	5,976,000	19,300	5,164,000	17,500
24 hours after injection	5,856,000	13,700	5,600,000	12.500

PERSISTENCE OF ACRIFLAVINE IN THE CIRCULATION

(a) Bactericidal Determinations.—Determinations of the following character were made in an effort to ascertain the degree of bactericidal action conferred upon the serum of rabbits by the intravenous injection of acriflavine. To this end rabbits were injected intravenously with the dyestuff and 15 minutes after the injection specimens of blood were taken for measuring the bac-

tericidal effect upon Bacillus typhosus *in vitro*, the test being performed according to the looped pipette method of Wright. The result of a preliminary experiment of this type showed that after a single injection of 1 c.c. of the dyestuff in a concentration of 1:100 the killing power of the serum was no greater after the lapse of a 15-minute interval than was that of the normal serum before the injection. Further tests of this type were performed with Staphylococcus aureus and repeated injections of the dyestuff were given in order to produce, if possible, a cumulative effect of the dye. Four injections of the maximal tolerated doses were given at 20-minute intervals. Blood for testing was withdrawn before the first injection and at intervals of one-half, one, and two hours following the last injection. Here again all of the titrations indicated that the injection of acriflavine even in large amounts had no effect in increasing the bactericidal properties of the serum. Apparently the dyestuff had been absorbed so rapidly by the tissues that it was impossible to produce and maintain an effective concentration within the blood stream.

The following experiment was performed with white mice, injections of acriflavine being given in an effort to prevent the mice from dying from pneumococcus septicemia after intraperitoneal injections of pure cultures of the pneumococcus. All of the injections of acriflavine were given intravenously with the maximal tolerated dose of the dye 0.4 c.c. of 1:5,000 solution. Table IV presents a protocol of an experiment of this type.

TABLE IV

MOUSE	VOLUME OF PNEUMOCOCCUS CULTURE INJECTED		RESULT
1.	1. c.c. of 1:1000 dilution	(approximately 1 M.L.D.)	D. 30 hrs.
2.	1. c.c. of 1:1000 dilution	0.4 c.c. acriflavine 1:500.	D. 27 hrs.
3.	0.5 c.c. of 1:100 dilution	(approximately 5 M.L.D.)	D. 24 hrs.
4.	0.5 c.c. of 1:100 dilution	0.4 c.c. acriflavine 1:500.	D. 30 hrs.
5.	1. c.c. of 1:100 dilution	(approximately 10 M.L.D.)	D. 24 hrs.
6.	1. c.c. of 1:100 dilution	0.4 c.c. acriflavine 1:500.	D. 24 hrs.
7.	0.5 c.c. of 1:10 dilution	0.4 c.c. acriflavine 1:500 (50 M.L.D.)	D. 27 hrs.
8.	1. c.c. of 1:10 dilution	0.4 c.c. acriflavine 1:500 (100 M.L.D.)	D. 24 hrs.
9.	1. c.c. of sterile saline	0.4 c.c. acriflavine 1:500.	Lived.

This experiment shows that the largest dose of acriflavine compatible with life when injected intravenously in white mice does not protect them in any degree from a fatal septicemia when produced by intraperitoneal injections of the pneumococcus.

CONCLUSIONS

Acriflavine, in a concentration of 1:2,000 or lower in physiologic saline, has no agglutinative action upon human erythrocytes.

In vitro, acriflavine in concentrations greater than 1:8,000 in saline is lytic for human erythrocytes. *In vivo*, acriflavine injections to the amount of 0.1 gram per kilogram of body weight (rabbit) induces a slight but transitory reduction in the number of erythrocytes, and a considerable increase in the number of leucocytes.

A single intravenous injection, or multiple injections, of the tolerated dose does not lead to an increase in the bactericidal action of the serum when tested against *Staphylococcus aureus.*

White mice injected simultaneously with the tolerated dose of acriflavine and the minimal lethal dose of virulent pneumococci are not protected by the dyestuff.

BIBLIOGRAPHY

[1]Bohland, K.: Deutsch. med. Wchnscr., Leipz. 7. Berl., 1919, xlv, 797.
[2]Bohland, K.: Mediz. Klinik, Berl., 1919, clii, 1173.
[3]Browning, C. H.: Lancet, London, 1917, ii, 436.
[4]Browning, C. H.: Ibid, 1917, ii, 621.
[5]Browning, C. H., Cohen, and Gulbransen, R.: Jour. Path. and Bacteriol., Cambridge, 1919-20, xxiii, 124.
[6]Browning, C. H., Gulbransen, R., and Kennaway, E. L.: Ibid, 1919-20, xxiii, 106.
[7]Browning, C. H., Kennaway, E. L., Gulbransen, R., and Thornton, L. H.: Brit. Med. Jour., 1917, i, 73.
[8]Browning, C. H., and Ligat, D.: Lancet, London, 1917, ii, 766.
[9]Carslaw, R. B., and Templeton, W.: Ibid, 1918, i, 634.
[10]Davis, E. G.: Nebraska Med. Jour., Norfolk, Neb., 1920, v, 19.
[11]Davis, E. G., and Beck, G. H.: Jour. Urol., Baltimore, 1921, v, 215.
[12]Davis, E. G., and Harrell, B. E.: Ibid, 1918, ii, 353.
[13]Davis, E. G., and White, B. E.: Ibid, 1918, ii, 299.
[14]Drummond, H., and McNee, J. W.: Lancet, London, 1917, ii, 640.
[15]Fleming, A.: Ibid, 1917, ii, 341.
[16]Fleming, A.: Ibid, 1917, ii, 508.
[17]Hewlett, R. T.: Lancet, London, 1917, ii, 493.
[18]La Favre, H. B.: U. S. Naval Med. Bull., Washington, 1919, xiii, 858.
[19]Morgan, W. P.: Lancet, London, 1918, i, 256.
[20]Ruhnau, K.: Therap. d. Gegenw., Berl., 1920, lxi, 220.
[21]Savery, H. M.: Brit. Med. Jour., London, 1918, ii, 283.
[22]Stowell, T. E. A.: Ibid, 1919, i, 244.
[23]Rep. Counc. on Pharm.: Jour. Am. Med. Assn., Chicago, 1919, lxxiii, 1443.

A SUBSTITUTE FOR CURARE IN CLASSROOM EXPERIMENTS*

By Nathaniel Kleitman, M.A., Ph.D., and Robert A. Crawford, S.B., Chicago, Ill.

DURING the war, when it was impossible to obtain curare, Dr. Luckhardt inquired of the Botanical Raw Products Committee of the National Research Council whether a substitute for curare could not be obtained in this country. He was informed "that among other plants which yield alkaloids producing physiological effects similar to curare, the most important are four species of Delphinium which are native to the western United States and northern Mexico, and it is entirely possible that supplies of this alkaloid could be obtained." Even after the war it was difficult to procure potent curare, and at the advice of Dr. Luckhardt we undertook the study of the effects of extracts prepared from mixed larkspur seeds, (Delphinium Consolida), readily obtainable from any seed dealer.

The seeds are dark gray, irregularly tetrahedral in shape, about one by two by one millimeters in size. We thoroughly ground them up in a coffee

*From the Hull Physiological Laboratory of the University of Chicago.
Received for publication, September 6, 1923.

mill, and first extracted them with ether by means of a Soxhlet extraction apparatus. The ether was allowed to evaporate and there remained a thick nondrying oil which we employed in a number of tests on frogs, albino rats, and dogs. Fifty grams of seeds would yield about 25 c.c. of oil. After the ether extraction the ground seeds were extracted with 95 per cent alcohol, either in the cold or by slightly warming. A few experiments were made with the alcohol extract. After the alcohol extraction, and in certain cases directly after the ether extraction, the seeds were further treated with a 5 per cent solution of sulphuric acid, usually about 350 c.c. of acid to 50 grams of seeds. The alcohol and acid extracts were likewise tested on frogs, albino rats, and dogs.

When injected into the ventral lymph sac of a frog, weighing between 35 and 45 grams, minute quantities of the oily extract produced paralysis in a very short time. In from 1.5 to 7 minutes the frog lost control of its equilibrium, and on pithing the animal and stimulating the sciatic nerve by means of a faradic current no contractions of the gastrocnemius could be observed. The muscle itself, however, readily responded to electrical stimulation. On several occasions we performed the well-known Claude Bernard experiment, tying off the circulation to one leg, and found that the nerve endings on that side were not paralyzed by the extract injected into the frog. We have performed a large number of these tests, and on the average 0.5 c.c. of the ether extract (corresponding to 1 gram of seeds) per 100 grams of frog will paralyze the motor nerve endings in the course of a few minutes. The alcohol and acid extracts also contain the active principle or principles, the amount of the extract necessary to produce the above results varying with the quantity of alcohol or acid used for the extraction.

We also injected these extracts intraperitoneally into white rats weighing from 150 to 250 grams. A 250 gram rat received 1 c.c. of the ether extract and went into convulsions in 35 minutes. It died 3 minutes later, and neither the sciatic nor the phrenic nerve was paralyzed. Similar results were obtained with the acid and alcohol extracts. To avoid the irritating effects of the sulphuric acid, the extracts were, in certain cases, neutralized by the addition of solid sodium bicarbonate, but the result was the same. The animals usually showed a labored respiration as a result of these injections, but caffeine failed to save them.

In our experiments on dogs, under ether or veronal anesthesia we found that 0.5 c.c. of the ether extract, injected intravenously, was sufficient to kill an animal weighing 8 to 10 kilos in the course of a few minutes. Death resulted from the paralysis of the respiration, but as in the case of the rats the excitability of the skeletal nerve endings was not abolished. In a number of tests when breathing stopped and the blood pressure began to fall, we instituted artificial respiration which resulted in recovery. After a while the animal would begin to breathe spontaneously, and after a suitable interval of time another injection of 0.5 c.c. of the ether extract again resulted in the cessation of respiration. By repeated resuscitation we were able to

inject 2 to 3 c.c. of the ether extract before death resulted from the paralysis of the circulation. Stimulation of the peripheral end of the cut vagus, before and after the injection, showed that the extract had a slight atropine-like action. Circulatory paralysis with an abrupt fall of the blood pressure after large doses of the ether extract took place even after both vagi had been sectioned, and since the vagal endings lost their irritability, the extract apparently acted on heart muscle directly.

Acid extracts have an action similar to the ether extract, and in some cases we were able to produce the typical effect by intraperitoneal injections of larger doses. But no matter how many injections we made and how long it took to kill the animal, we were never able to produce paralysis of the skeletal nerve endings.

From Delphinium consilada, Heyl[1] in 1902 prepared a substance marketed by E. Merck under the name of *Delphocurarin*. The pharmacological properties of this substance have been investigated by Lohmann,[2] Schiller,[3] Krehichkowsky,[4] and Kahn.[5] It is agreed that on the cold blooded animal this mixture of alkaloids exercised an action similar to curare; there is, however, some uncertainty in regard to its action on the mammal. A great deal of work can be found in the literature on the isolation and action of the various alkaloids obtained from the corresponding European plant, Delphimium Staphisagra, while the identification of the alkaloids from larkspur has been almost entirely neglected. One usually finds the statement that inasmuch as larkspur is almost identical to stavesacre, the alkaloids in larkspur can be assumed to be identical to those of stavesacre. As our work with a crude ether extract confirms the work of other investigators, it is our opinion that further investigation should be carried out on the alkaloids of the American Delphinium.

Since it is difficult to get curare that actually works, we advise the use of the ether extract of larkspur seeds in the Claude Bernard experiment, which is usually included in the laboratory courses in physiology. It was given a thorough test in the performance of the above experiment by a class of medical students, and the results were very satisfactory. It cannot be used, however, in mammalian experiments when a curare-like effect is desired, and its action on the vagus endings is too feeble to make it of value as a substitute for atropine.

REFERENCES

[1]Heyl, G.: Südd. Apoth.-Zeit., 1902, xliii, No. 28, 29, 30. (Abst. can be found in Chem. Centralbl., 1903, I, 1188).
[2]Lohmann, A.: Pflüger's Arch., 1902, xcii, 473.
[3]Schiller, V.: Arch. f. Anat. u. Physiol., Physiol. Abst., 1904, p. 248.
[4]Krehichkowsky, K.: Arch. internat. de Pharmacol., xviii, 65.
[5]Kahn, R. H.: Pflüger's Arch., 1916, clxiv, 428.

A METHOD FOR THE DETERMINATION OF BLOOD UREA NITROGEN*

By Walter G. Karr, Ph.D., Philadelphia, Pa.

INTRODUCTION

In recent years hemochemistry has assumed an important place in laboratory diagnosis in all modern hospitals. Of the blood constituents determined none has greater importance in routine work than the urea nitrogen in certain diseases, especially kidney dysfunction. Uric acid may possibly be retained first in beginning nephritis[1] but it does not have the range of variation in following the course of nephritis that is observed in urea nitrogen. The determination of blood urea nitrogen is preferable to that of nonprotein nitrogen, as the latter includes constituents concerning whose metabolism we know little and which vary with the protein precipitant used.[2] The proportional increase of urea nitrogen in nephritis is usually greater than that of nonprotein nitrogen, often rising to 75 or 80 per cent of the latter.

Most of the present methods for the quantitative estimation of blood urea nitrogen depend upon the conversion of the urea to ammonium carbonate by the enzyme urease. After alkalization the ammonia is collected by aeration or distillation and measured by titration or colorimetrically with Nessler's reagent. The method of Van Slyke and Cullen,[3] with certain modifications, is satisfactory and accurate for routine work. It is, however, time consuming and there are factors, especially the enzyme and the aeration, which must be carefully controlled to make it practical for the numerous routine analyses performed in large hospital laboratories. Folin and Wu[4] in their system of blood analysis have suggested four methods for the determination of blood urea nitrogen in the tungstic acid filtrates. Of these the method of conversion with urease and distillation with borax seems most desirable. The distillation in this method makes it very inconvenient and tedious for a technician to complete numerous determinations. The difficulties with the above methods have caused many to use the determination of nonprotein nitrogen in preference to that of urea for routine diagnosis. It is highly desirable, therefore, to have a shorter method for blood urea nitrogen estimations which will make it convenient for use, especially in those laboratories using the Folin and Wu system of blood analysis.

EXPERIMENTAL WORK

When blood filtrate from protein precipitation by tungstic acid is digested with phosphate buffer solution and a fairly clear urease solution, and, after dilution, Nesslerized, there is obtained a clear colored solution. This color differs from that developed by Nessler's solution and pure ammonium salts in having a greenish tint. An artificial standard may be prepared which is an excellent match to this greenish orange color. This standard consists of a mixture of iron, cobalt and nickel chlorides in proper proportions. The arti-

*From the Laboratory of Biochemistry of the Philadelphia General Hospital and the Graduate School of Medicine, University of Pennsylvania.
Received for publication, June 28, 1923.

ficial color is standardized against the color developed, as indicated above, from a known urea solution. This solution is placed in the colorimeter with the artificial standard and the latter diluted until they match. With a given urease preparation the amount of color developed is very constant from time to time, and the dilution of the standard is practically always the same. Although the reading of the Nesslerized solution does not change for a number of minutes, it is desirable for routine practice to Nesslerize one tube at a time and read it directly, which is usually about one minute after the reagent is added. The color does not increase in intensity to any appreciable extent until turbidity gradually develops after several minutes. It is of value in this determination to have a uniform enzyme preparation. This is made according to the directions of Folin[5] with the exception that a mechanical stirrer or shaker is used for a definite length of time, generally fifteen minutes. Since this modification has been introduced no trouble has been experienced with inactive urease preparations which occasionally resulted when the extraction was made by hand. This enzyme solution has only a slight cloudiness and successive preparations are uniform in this respect. The urease paper as described by Folin[5] is especially adapted to this method and has the advantage of being much more permanent than the urease solution. This is of value where only occasional determinations are made. The color is more orange, necessitating an artificial standard of slightly different composition.

SOLUTIONS.—*Urease Solution*—prepared according to the directions of Folin.[5] "Wash about 3 gm. of permutit in a flask once with 2 per cent acetic acid, then twice with water; add 5 gm. of fine Jack bean meal and 100 c.c. of 15 per cent alcohol (16 c.c. of ordinary alcohol plus 84 c.c. water). Shake gently but continuously for 10 to 15 minutes, pour on a large filter and cover with a watch glass." Use a mechanical stirrer or shaker instead of shaking by hand.

Urease Paper—prepared according to the directions of Folin.[5] Shake 15 gm. of Jack bean meal with 5 gm. permutit and 100 c.c.* of 15 per cent alcohol for 10 to 15 minutes. Filter through a pleated filter and place the clear filtrate into clean, flat bottomed dishes. Draw pieces of ammonia-free filter paper through the solution and hang up to dry. When dry cut into strips about one inch long and one-half inch wide. The size is convenient to drop into test tubes. The preparation of Jack bean meal in use in this laboratory contains only a trace of ammonia. Where such preparations are found one may dispense with the permutit and simply make the extraction with 15 per cent alcohol.

Phosphate Buffer Solution—prepared according to the directions of Folin.[5] Dissolve "14 gm. of sodium pyrophosphate ($Na_4P_2O_7$, $10H_2O$) in enough half normal phosphoric acid to make 100 c.c. The half normal phosphoric acid is made by diluting 20 c.c. of 85 per cent phosphoric acid to one liter and titrating 5 c.c. with tenth normal alkali and phenolphthalein as indicator, to a faint pink color. On the basis of this titration dilute the acid to a substantially correct half normal solution."

Stock Urea Solution—0.1286 gm. urea dissolved in water and diluted to 200 c.c. One c.c.=0.3 mg. nitrogen.

*There is an apparent typographical error in this reference with regard to the number of c.c. of 15 per cent alcohol used.

Standard Urea Solution—5 c.c. of the stock urea solution diluted to 100 c.c. with water. Five c.c.=.075 mg. nitrogen.

Artificial standard solution:

For use with urease solution:

5 c.c. 10 per cent $FeCl_3.6H_2O$ (Add 0.5 c.c. conc. HCl to 100 c.c.)
1 c.c. 10 per cent $CoCl_2.6H_2O$
1.5 c.c. 10 per cent $NiCl_2.6H_2O$

dilute as indicated later.

For use with urease paper:

5 c.c. 10 per cent $FeCl_3$
1.5 c.c. 10 per cent $CoCl_2$
0.75 c.c. 10 per cent $NiCl_2$

diluted as indicated later.

Nessler's Solution—prepared according to the direction of Folin.[5] "A stock solution of double iodide is best prepared as follows: Transfer 150 gm. of potassium iodide and 110 gm. of iodine to a 500 c.c. Florence flask; add 100 c.c. of water and an excess of metallic mercury, 140 gm. to 150 gm. Shake the flask continuously and vigorously for 7 to 15 minutes, or until the dissolved iodine has nearly all disappeared. The solution becomes hot. When the red iodine solution has begun to become visibly pale, though still red, cool in running water, and continue to shake until the reddish color of the iodine has been replaced by the greenish color of the double iodide. This whole operation usually does not take more than 15 minutes. Now separate the solution from the surplus mercury by decantation and washing with liberal quantities of distilled water. Dilute the solution and washings to a volume of two liters. If the cooling was begun in time, the resulting reagent is clear enough for immediate dilution with 10 per cent alkali and water." Nessler's solution is prepared by adding 150 c.c. of the above reagent to 700 c.c. 10 per cent sodium hydroxide and 150 c.c. water. It is very important to have the solution free from carbonate. The sodium hydroxide is prepared by adding an equal weight of water to stick sodium hydroxide and after solution allowing the insoluble sodium carbonate to settle. It is titrated and diluted to 10 per cent. Nessler's solution is protected from the carbon dioxide of the air by a soda lime tube.

In case the Nessler's reagent is withdrawn from its container through a tip exposed to the air this should be protected by covering with a small tube. Too much emphasis cannot be laid on the care of the Nessler's reagent. Do not leave the solution in contact with rubber tubing.

Dilution of the Standard.—To each of two or three ordinary test tubes transfer 5 c.c. of the standard urea solution containing .075 mg. nitrogen. Add 1 or 2 drops of the buffer solution and 1 c.c. urease or one strip of urease paper. Digest at 45 to 50° for 5 minutes. Transfer quantitatively to another set of test tubes washing with two portions of about 5 c.c. of water. (The latter test tubes are graduated at 22.5 c.c. and 25.0 c.c. The transfer is necessary because of the destruction of the urease by the mercury in case the same tubes are used for both operations.) Dilute to the 22.5 c.c. mark with water and add Nessler's solution to the 25 c.c. mark. Mix by inversion. Place in the colorimeter and set at 15 mm. The artificial standard is prepared as indicated

above and diluted until it reads 15 mm. when placed in the opposite cup. The standard is made in a 50 c.c. graduated cylinder and the degree of dilution is determined by trial. Other standard urea tubes are Nesslerized in the same manner and the amount of dilution checked. After the dilution of the standard has been determined it is placed in a well stoppered bottle and kept for subsequent determinations. It has been our experience covering several hundred urea determinations that the reading of the standard is nearly always not more than 0.2 to 0.3 mm. different from the reading of the color developed from the standard urea solution. It is recommended, however, that at least one standard urea solution be read against the standard with each set of urea determinations. This routinely checks the activity of the urease preparation in addition.

Determination of Blood Urea Nitrogen.—Transfer 5 c.c. portions of the blood filtrate from tungstic acid protein precipitation (Folin and Wu[4]) to ordinary test tubes and proceed with the digestion and Nesslerization exactly as directed above with the standard urea solutions. Having the standard in the colorimeter, place the unknown at 15 mm. and read the standard as soon as convenient after Nesslerization, generally about one minute. In case the standard reads above 30 mm. repeat the determination, using appropriately less blood filtrate. In case of high urea the Nesslerized solution assumes a deep orange color, clouds immediately and cannot be used. It is then necessary to repeat the determination, using a smaller amount of the filtrate as experience indicates by the color developed. The time consumed in the test is so short that it is no inconvenience to repeat the determination.

Calculation: X = mg. urea nitrogen per 100 c.c. blood
S = reading of the standard
Set the unknown at 15 mm.

$$\text{Then } X = \frac{S}{15} \times 0.075 \times \frac{100}{0.5} = S$$

Thus the above arrangement eliminates all mathematical calculation, as the milligrams of urea nitrogen are read directly on the colorimeter. Where smaller amounts of filtrate are used, the above calculation is modified accordingly. The method of setting the unknown and reading the standard was introduced into this laboratory by Dr. W. H. Stoner and greatly simplifies all routine calculations used in colorimetry.

Table I gives results of analyses of blood by the Van Slyke and Cullen[3] urease and aeration method on whole blood, the Folin and Wu[4] urease and distillation method on blood filtrate and the present method. The data represent averages of check determinations.

Folin and Wu suggest that direct Nesslerization is impracticable because of the interference of peptones and amino-acids. These constituents are very constant with both normal and pathologic bloods, and any slight interference which they exert is similar for all bloods and does not in any way influence the value of the data for clinical diagnosis.

This method may be used with other body fluids providing the amount of precipitating reagent is decreased so that the excess tungstic acid does not interfere with the activity of the urease.

TABLE I

BLOOD NO.	MILLIGRAMS UREA NITROGEN PER 100 C.C. BLOOD		
	PRESENT METHOD	FOLIN AND WU	VAN SLYKE AND CULLEN*
A	13.3	13.2	
B	15.2	15.5	16
C	82.	80.	
D	20.6	20.2	
E	18.1	17.9	
F	13.7	13.6	14
G	13.5	13.2	
H	13.8	13.6	
I	13.3	13.2	
J	29.5		30
K	46.	45.	
L	135.		138
M	78.		79
N	57.		58
O	23.5		24
P	34.2		33

*The author is indebted to Alice B. O'Neill of this laboratory for the analyses reported in this column.

The usual experiments were performed by adding known amounts of urea to both whole blood and filtrate. The recovery was theoretical within experimental error in all cases. The color was developed from known amounts of urea (0.04 to 0.15 mg. nitrogen) and read with the artificial standard. The reading was proportional to the amount of urea nitrogen within the above limits.

SUMMARY

Blood urea nitrogen is determined in the tungstic acid filtrate by urease conversion, Nesslerization, and colorimetric comparison without removal of the ammonia from the digestion mixture. An artificial standard solution is used which is composed of a mixture of iron, cobalt, and nickel chlorides, previously standardized against a known urea solution. The results of estimations by this method are accurate, comparing favorably with those of the Van Slyke and Cullen, and Folin and Wu methods. The method is simple and numerous determinations can be completed in short time. Hence, the method should be of particular value to the hospital laboratory where the great volume of routine requires simplicity of manipulation and calculation, and conservation of time and apparatus.

NOTE: Since sending this manuscript for publication, experience with over 3000 urea nitrogen determinations by this method indicates the following points should be emphasized: The urease paper is much more convenient and preferable to the urease solution. The artificial standard occasionally becomes cloudy, due, perhaps to deposition of ferric salts, and should of course then be freshly prepared. When only a few determinations are to be made, and urease paper is used, it is possible and perhaps preferable to read the unknown against the color developed from the standard urea solution instead of the artificial standard. (W. G. K.)

BIBLIOGRAPHY

[1]Myers, Fine and Lough: Arch. Int. Med., 1916, xvii, 570.
[2]Hiller and Van Slyke: Jour. Biol. Chem., 1922, liii, 253.
[3]Van Slyke and Cullen: Jour. Am. Med. Assn., 1914, lxii, 1558.
[4]Folin and Wu: Jour. Biol. Chem., 1919, xxxviii, 81.
[5]Folin, O.: Laboratory Manual of Biological Chemistry, N. Y., 3d edition, 1922.

LABORATORY METHODS

THE STANDARDIZATION OF PITUITARY EXTRACT BY THE OXYTOXIC METHOD AND DESCRIPTION OF APPARATUS FOR CONTROLLING THE TEMPERATURE*

By Edward E. Swanson, Indianapolis, Ind.

RECENTLY, there has been a great deal of discussion regarding the choice of a method for the standardization of pituitary extracts. According to Dudley[1, 2] the posterior lobe contains two distinct principles, the pressor activities of which are quite different. This confirms the writer in the opinion he has long held, that the pressor method is not suitable, except, perhaps, for the testing of those pituitary products which are especially designed for surgical use. For extracts to be used in obstetric practice, the oxytoxic method of testing certainly seems to be the more logical, and, if certain precautions are observed, will be found to be accurate.

Success with the bio-assay of pituitary extracts by the oxytoxic method is dependent on the following four factors:

I. Satisfactory apparatus for maintaining a uniform temperature.
   A. Apparatus.
   B. Effect of temperature on the uterus.
II. Locke Ringer solution.
III. Selection of a suitable uterus.
IV. Suitable standard substance for comparison.

I. SATISFACTORY APPARATUS FOR MAINTAINING A UNIFORM TEMPERATURE

A. *Apparatus.*—The main features of this apparatus are essentially the same as those described by Dale and Laidlaw,[3] but certain improvements have been made which increase the accuracy of the method and number of tests possible in a given time. Dale and Laidlaw[3] in their original paper, describe their apparatus as consisting of a water-bath kept at uniform temperature by a copper rod passing through the bath, the rod being heated by a Bunsen burner. By moving this burner along the rod, the temperature of the bath can be kept at 38° to 39° C. Roth[4] used a modification of the above method in which a constant flow of heated water is maintained through the bath which contains two aspirator bottles of 500 c.c. capacity filled with Locke solution. Eckler[5] devised an apparatus which is almost entirely made of glass. The glass bath, essentially being a bell jar inverted, contains glass

*From the Lilly Research Laboratories, Pharmacology Department, Indianapolis, Ind.
Received for publication, October 9, 1923.

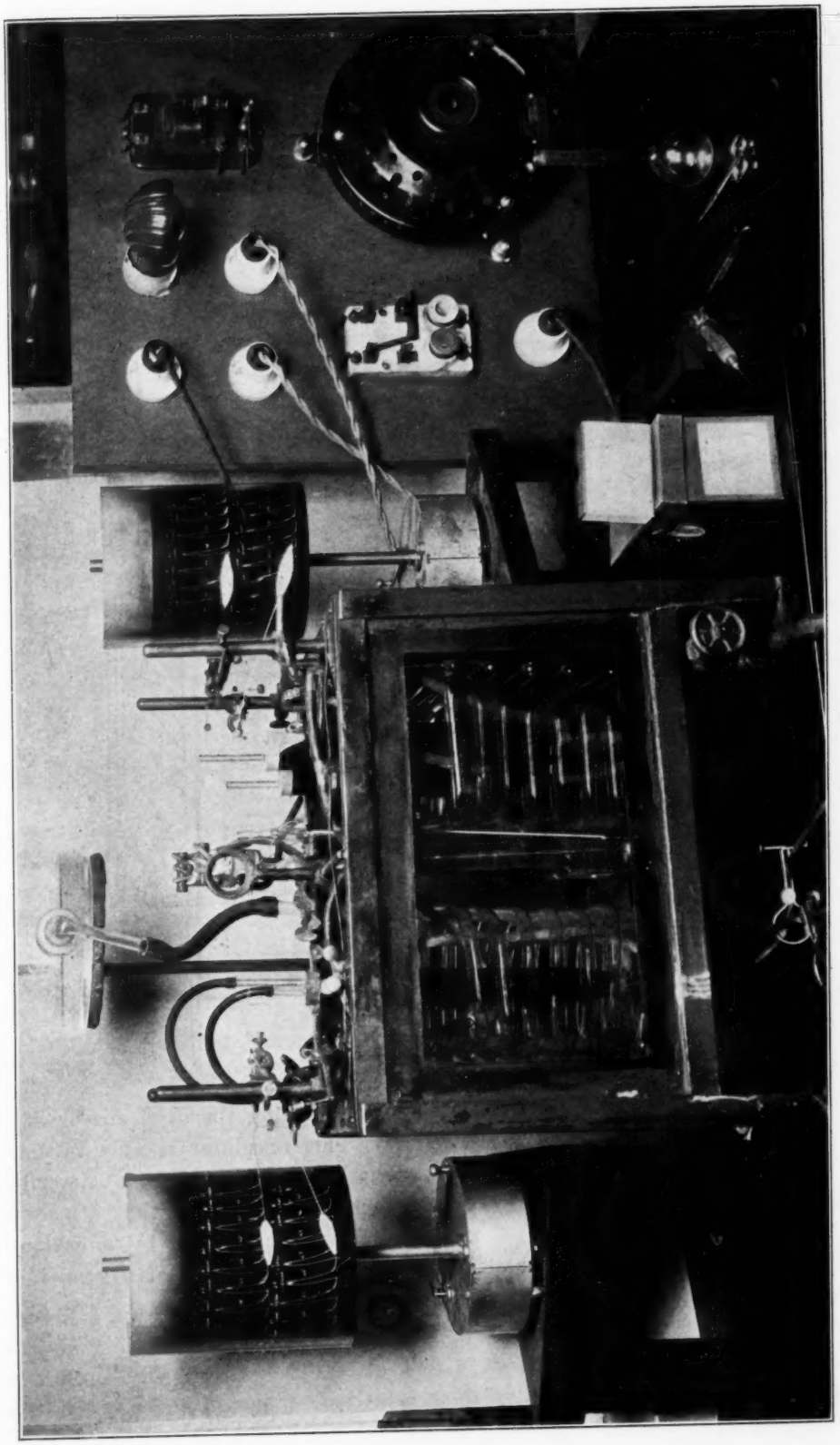

Fig. 1.

coils and tubes, which are filled with Locke solution, and kept at a constant temperature by a carbon light immersed in the bath. Two such units were set up and used simultaneously in order to utilize both horns of the uterus and to save time in check testing. Pittinger[6] describes an apparatus for maintaining a uniform temperature of the bath, which consists of an electric heater controlled by a thermoregulator, batteries, condensers, and relay. Pittinger[7] later describes another improvement of this same apparatus by

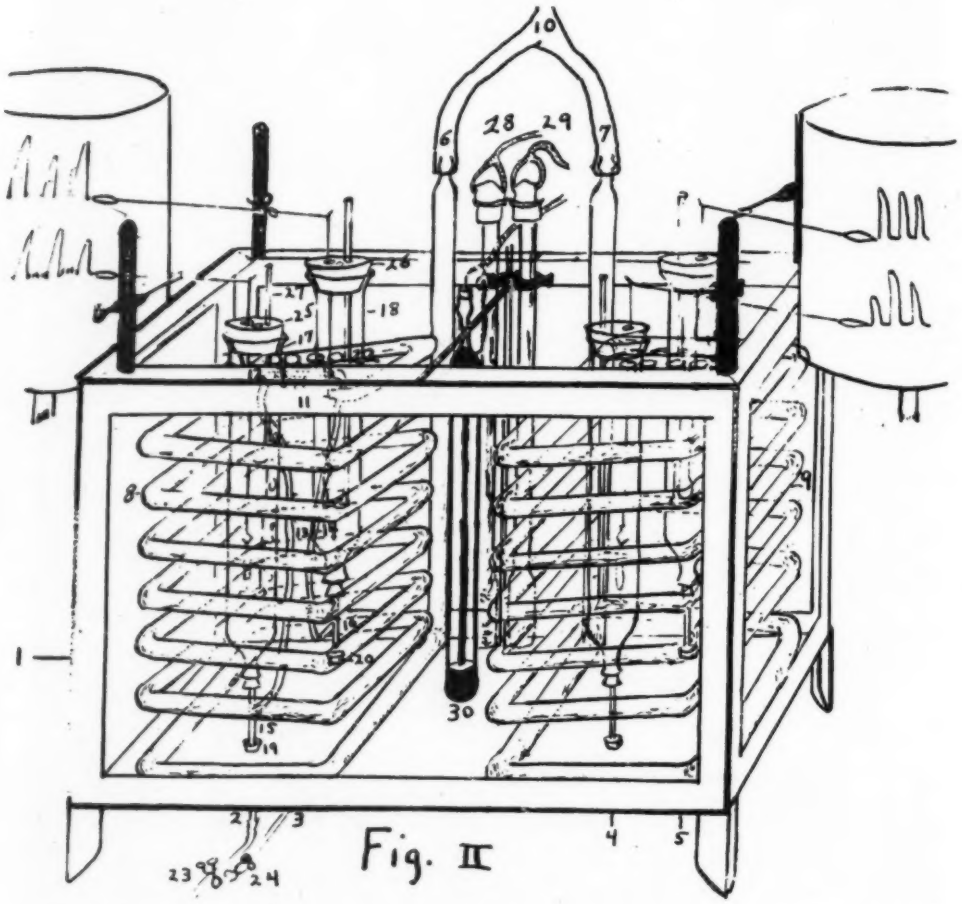

Fig. II

readjusting the toluol-mercury-regulator, and replacing the dry cells or batteries with an electric "toy transformer." This regulator is able to control the temperature to less than 1° C. variation. Burn and Dale[8] describe a method of fixing the glass chamber, which is held tightly to the bath by a packed gland, and a method of regulating the temperature by a carbon-filament electric glow lamp which can be moved in and out of a tunnel in the bottom of the bath to maintain a constant temperature. They also describe a new recording lever, which is a thin magnolium wire set on a circular slab of ebonite and carrying a delicate frontal writing point.

The apparatus here described increases the number of tests in a given

time, and automatically controls the temperature of the water in the bath within 0.2° C. variation.

In its present form (Fig. I) the apparatus consists of two units with which four tests may be made simultaneously.

The essential part of the two units is a water bath (1) Fig. II, with tubes, coils, etc. The bath is an oblong tank, which measures 18 inches in length, 12 inches in width, and 12 inches in depth. The frame, bottom and legs are made of brass, and the four sides are made of glass. It has four tubulatures (2, 3, 4, 5) at the bottom and two tubes (6, 7) at the top.

Two glass coils (8, 9) each of 500 c.c. capacity are set in the bath at each end, each coil surrounding two chambers and each chamber containing one horn of the uterus. The coils receive the Locke solution from a large reservoir (10) as needed, and this does not remain in the coils more than 20 to 30 minutes, thus avoiding the precipitation of the calcium salts. The solution from the reservoir enters the coils from below and flows from the bottom up to the top part of the coils. To the upper end of each coil a rubber tube (11) is attached. This rubber tube is joined to a T tube (12), each arm of which passes into rubber tubes (13, 14) which are attached to two T tubes (15, 16). One arm of each tube passes up through the lower stopper of the chamber (17, 18) and the other arm of each tube passes down through the stopper (19, 20) at the bottom of the bath. By means of pinchcocks (21, 22) the Locke's solution may be flowed through the coils from the reservoir into the chambers (17, 18) and the chambers emptied by the pinchcocks (23, 24).

The chambers containing Locke's solution, in which the isolated muscles are immersed, are of amber glass tubing about 10 inches long and 1 inch in diameter, and are graduated to hold 100 c.c. Each chamber is fitted at the top with a cork stopper (25) which has three holes, one for a glass tube (27) which conveys air to the muscle and serves to hold the lower end of the uterus, one for the thread from the uterus to the writing lever, and the third hole for introducing the test solution.

The water in the bath is maintained at a constant temperature by two electric heaters (28, 29), Fig. II. The heater (28) is a continuous one which keeps the temperature of the bath to about 35° C. The current through this heater is lowered or increased by a rheostat (Fig. III, position IV) depending upon the temperature of the laboratory. The heater (29) is an intermittent heater, regulated by a mercury-toluol regulator (30), which makes or breaks the current through heater, and maintains the temperature to either 37°, 38° or 39° C. as desired. Both heaters are controlled by a series of circuits on a switchboard.

The switchboard, Fig. III, which holds the equipment for controlling the current through the heaters (28, 29), is a slate slab about 24 inches square and 1 inch in thickness. Position I (Fig. III) is a socket for connecting the board to any 110 volt circuit, direct or alternating current. Position II is a double knife switch with fuses. Position III is a socket for connecting the continuous heater (28) 300 or 500 watt (110 volts). Position IV is a special C-H rheostat, 4.5 amperes, 110 volts, 85 ohms, in series with continuous

heater. Position V is a socket for connecting the intermittent heater (29) 75 watt (110 volts). Position VI is a socket for a 60 watt light, used as a resistance to reduce voltage for the circuit through the thermostat which is in series with the magnet coils in the relay. Position VIII is a relay used for making and breaking the circuit through the intermittent heater (29).

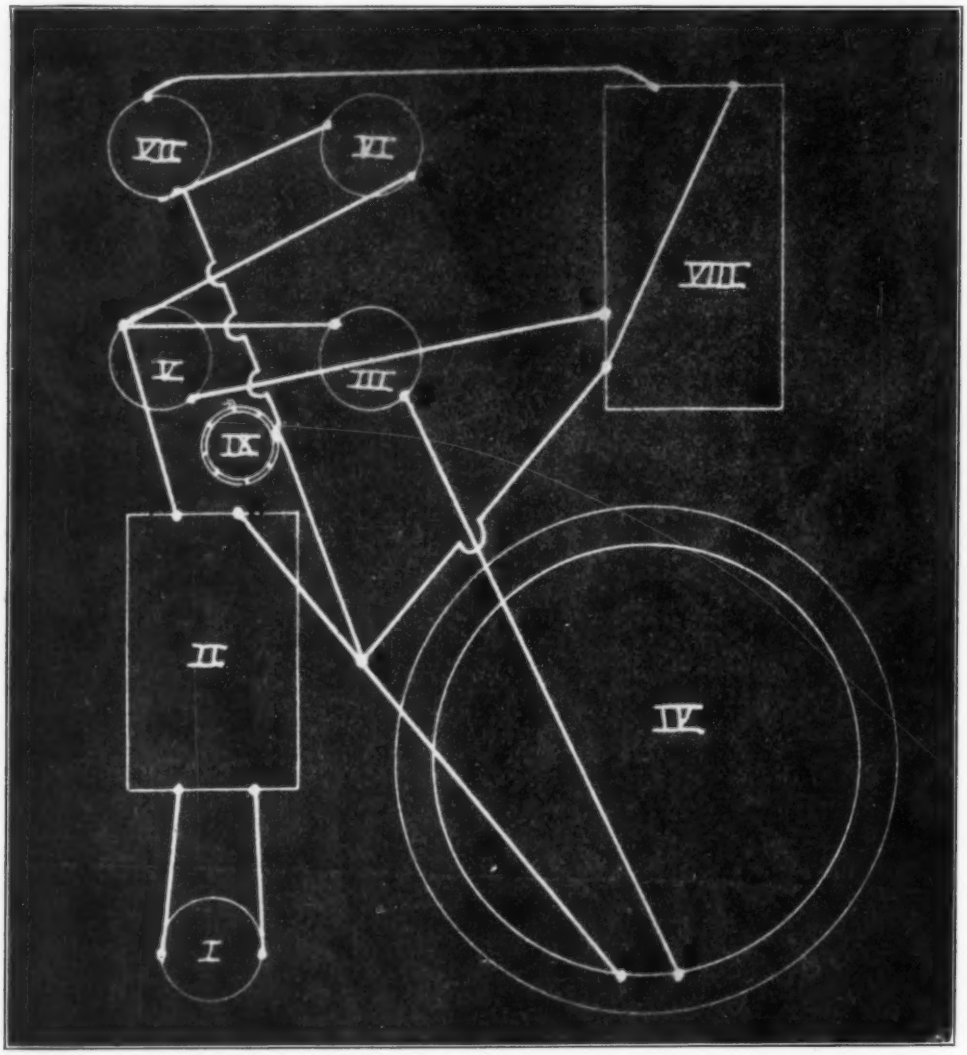

Fig. III.

Position IX is a resistance coil which furnishes a low voltage in the thermostat circuit.

The wiring of the switchboard may be studied by tracing the circuits in Fig. III. The current passing through the continuous heater (28) takes the path *A, B, C, D, E, F, G, H* and it may be lowered or increased by the rheostat. The current passing through the intermittent heater follows the path *A, B, J, K, L, G, H*. The circuit through the mercury-toluol regulator

(30) is *M, N, O, P, L, G, M,* while that through the electric light is *A, B, R, S, M, G, H* (also possible through *A, B, R, S, M, N, O, P, L, G, H*). When the temperature of the bath is below the standard temperature desired the mercury-toluol regulator does not permit the circuit *M, N, O, P, L, G, M,* and the intermittent heater is allowed to function. As soon as the standard temperature desired is exceeded, the mercury-toluol regulator closes the circuit *M, N, O, P, L, G, M* which magnetizes the core in the relay and draws the armature to it, thus breaking the circuit *A, B, J, K, L, G, H* through the intermittent heater. The voltage through the circuit *M, N, O, P, L, G, M* is about twelve volts, and is obtained by taking the drop in voltage across the resistance coil, Position IX, which is in series with the electric light. This wiring accomplishes the same purpose as Pittinger's "toy transformer," but it may be used with direct as well as alternating current.

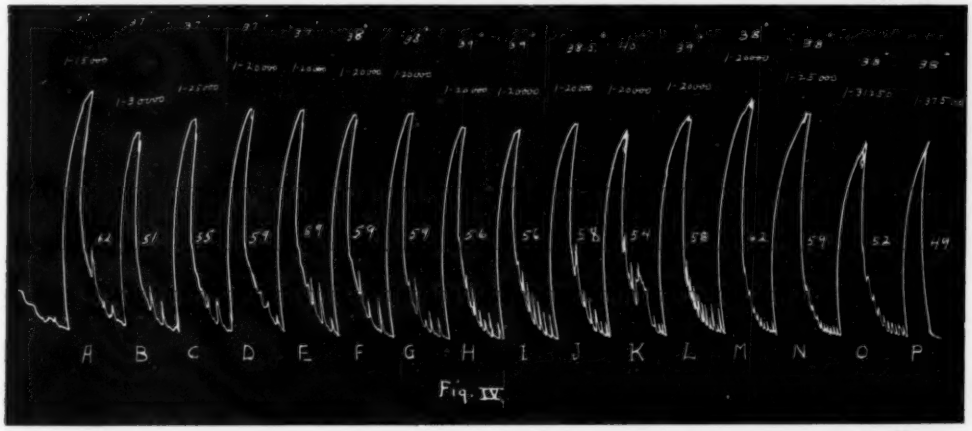

Fig. IX

The switchboard with this equipment can be easily moved, connected to any 110 volt circuit, and will maintain a constant temperature within 0.20° C. if efficiently stirred by bubbling air through the bath from the bottom.

For the purpose of giving a clear understanding of the technic and control of the apparatus a brief explanation will be given. The distilled water in the bath is first brought to the desired temperature by the heaters. While this is going on, Locke's solution may be made up and introduced into the reservoir, the dilutions of the extracts to be tested made, the drums smoked, needles threaded, etc. When the apparatus is adjusted, the pigs are decapitated, and the uterus isolated.

B. *The Effect of Temperature on the Uterus.*—The value of maintaining a constant temperature is essential in order to obtain the same size contractions with the same amount of pituitary extract. During the dependable period of a choice uterus, a variation in temperature of 0.5° C. will give a considerable difference in the size of contractions with the same amount of extract.

The *dependable* period of a select uterus is usually about two to four hours. There is a half hour to an hour at the beginning of an experiment,

the *adjustable* period, and an hour or more at the end, the *hypersensitive* period, when the contractions are not very reliable though they will give some idea of the strength of the sample. In some experiments the dependable period may be prolonged by raising the temperature 0.5° C. to 1° C. at the beginning of the hypersensitive period. A uterus with lively normal rhythm or contractions at a temperature of 37° C. may become less irritable, if the temperature is raised to 38° C. or 39° C. By comparing the tracings of many uteri at 37° C. and 38° C. the contractions, while not always equal,

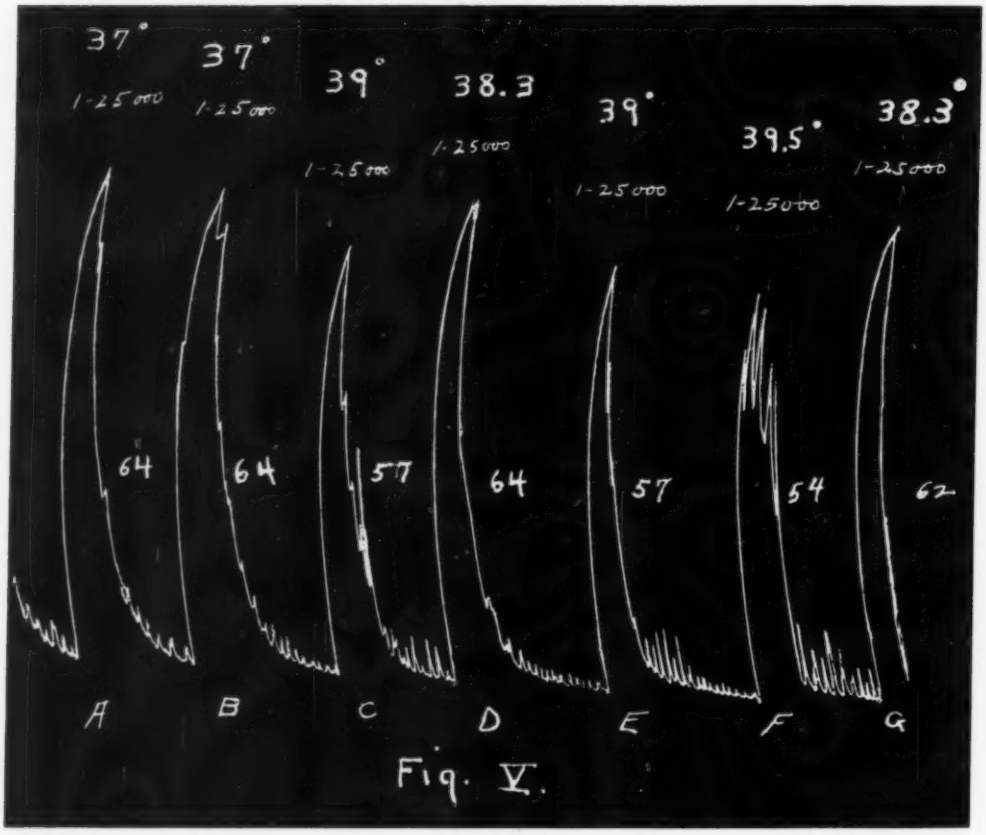

Fig. V.

show little variation with the same dose of pituitary extract. If the uterus is subjected to an increase in temperature of 1° C., that is to 39° C., the contraction will be smaller than at 37° C. or 38° C. with the same dose of pituitary extract. If the uterus is subject to a further increase of temperature, that is to 40° C., the contraction will be still smaller, with the same dose of pituitary extract.

Fig. IV represents a tracing obtained at various degrees of temperature. Contraction *A* represents a maximum contraction at 37° C.; *B* is half the dose of *A;* contractions *B, C* and *D* show a quantitative response at 37°; *D* and *E* show a check with equal amounts of drug; *F* and *G* are equal to *D* and *E* at 38°; *H* and *I* at 39° are less than *F* and *G* at 38°, which shows that

the uterus is less sensitive at the higher temperature. Contractions *J*, *K* and *L* show a difference at 38.5°, 40° and 39° respectively, and *M*, *N*, *O* and *P* show a quantitative response at 38°. In Fig. V contractions *A* and *B* are equal at 37°; *C* at 39° is less than *A* and *B*; *D* at 38.3° is equal to *A* and *B*, and *E*, *F* and *G* show a distinct difference at 39°, 39.5°, and 38.2° respectively. This uterus was sensitive to a difference in temperature of 0.5° C. at

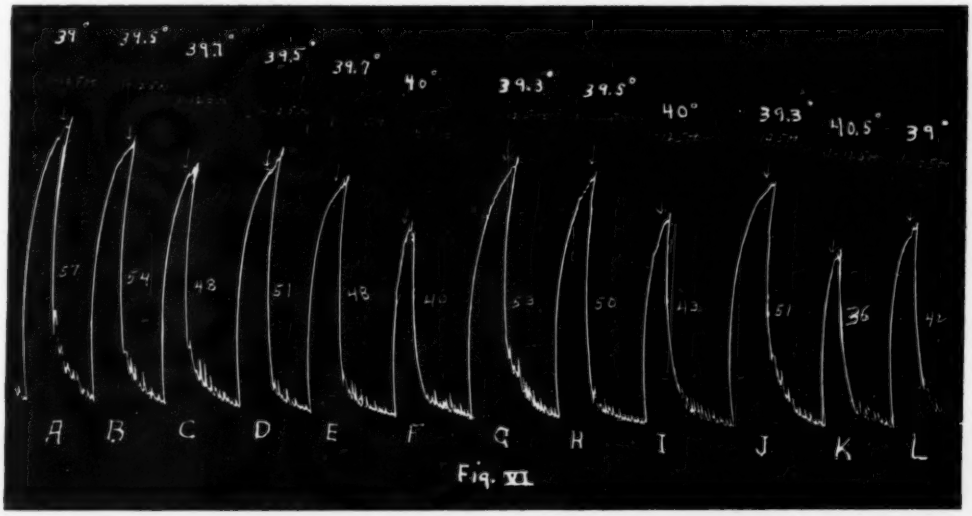

Fig. XI

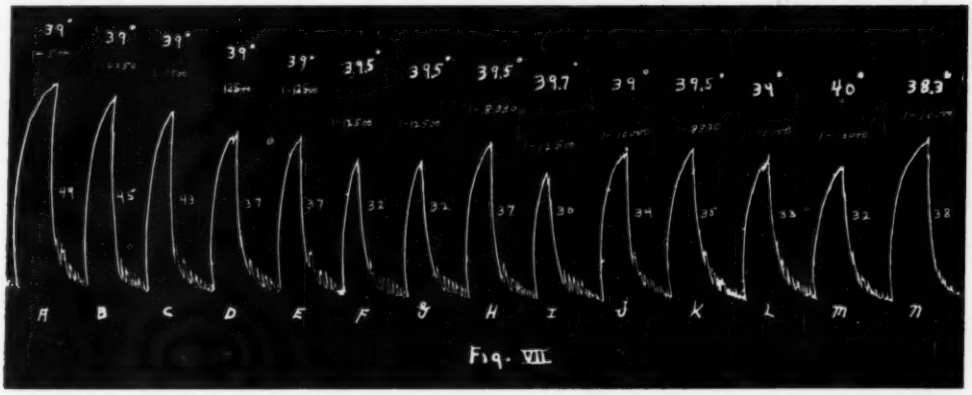

Fig. VII

37°, 38° and 39.5.° In Fig VI contractions *A*, *B* and *C* at 39°, 39.5° and 39.7° respectively, with equal doses measure 57 mm., 54 mm., and 48 mm.; likewise, *D*, *E* and *F* at 39.5°, 39.7°, and 40° measure 51 mm., 48 mm. and 40 mm. respectively; *G*, *H* and *I* at 39.3°, 39.5° and 40° measure 53 mm., 50 mm. and 43 mm. respectively, and finally, *J* and *K* at 39.3° and 40.5° measure 51 mm. and 36 mm., respectively. In Fig. VII contractions, *A*, *B*, *C* and *D* at 39° show a quantitative response with different size doses of pituitary extract, *E* checks contraction *D* at 39°; and *F* and *G* check at 39.5°. At *H* the dose was increased. *I*, *J*, *K*, *L* and *M* at 39.7°, 39°, 39.5°, 39° and 40°,

respectively, show a quantitative response with different size doses of the drug and changes of temperature.

Therefore, it is possible to observe from the above tracings, that there is a distinct difference in the size of contraction with equal amounts of drug at various temperatures; that the sensitivity of the uterus decreases as the temperature increases; and that a constant temperature is quite necessary in comparing an unknown sample with any standard solution or extract.

II. LOCKE RINGER SOLUTION

The composition of the Locke Ringer solution is the same formula as outlined by Dale and Laidlaw[3] and Burn and Dale[8] as follows:

NaCl	9.0	gm.
KCl	0.42	gm.
$CaCl_2$	0.24	gm.
$NaHCO_3$	0.5	gm.
Dextrose pure	0.5	gm.
Mg. Cl_2	0.005	gm.
Water distilled	1000	c.c.

The distilled water should be freshly distilled and all chemicals should be of the highest purity, particularly sodium chloride and dextrose. Pittinger and Quici[9] have found that sodium chloride in large crystals produces a less irritable uterine muscle than sodium chloride in small crystals. Kochman[10] has recommended a reduction in calcium chloride until the spontaneous movements of the uterus have stopped. In a recent communication, Smith and McClosky[11] reported that by washing the oxygen through a solution of 2 per cent sodium bicarbonate the irritability of the uterus is decreased. A method of segregating guinea pigs and selection of choice uteri has given results so gratifying, that the writer has seen no necessity in changing the above Locke Ringer mixture.

III. SELECTION OF A SUITABLE UTERUS

The selection of a uterus, regarding size and physical appearance, has been the subject of much discussion. For the last three years the problem of obtaining a choice uterus has been made much easier by segregating the females from the males. At a young or weaning age, the more healthy, rugged looking ones are separated. The same plan has been followed by Burn and Dale[8] and Smith and McClosky.[11] Some workers prefer pigs of 250 gm. to 375 gm. weight, others prefer pigs of 175 gm. to 250 gm. weight. The writer finds that pigs of 225 gm. to 300 gm. weight give a greater number of suitable uteri. The larger pigs have muscles which are more irritable, hypersensitive and prone to greater normal contractions or increased rhythm. Pigs of less than 200 gm. weight usually have uterine muscles too delicate, stringy and slow to respond to pituitary extract, and the dependable period is short. The best uterine horns are obtained from virgin pigs. A muscle that is in estruation, the vulva watery and swollen, the fallopian tube thick and reddish, does not give suitable results. A choice uterus is pale

in appearance, slender, and the circular muscles relaxed. By segregating the pigs, the worker should find no difficulty in obtaining a suitable uterus.

IV. SUITABLE STANDARD SUBSTANCE FOR COMPARISON

The question of a suitable standard for comparing a preparation of unknown strength has been the subject of some controversy. Dale and Laidlaw[3] in their original article suggested some preparation from the posterior portion of the pituitary gland; later Roth[4] suggested the use of histamine, which is official in the U.S.P. IX. Still later, Spaeth[12] found that histamine is not stable, and for other reasons is not suitable for a standard. He thought that potassium chloride would be a more satisfactory standard. Recently, Burn and Dale,[8] and later Nelson[13] proved experimentally that both histamine and potassium chloride are pharmacologically different in action from pituitary extract on the uterine muscle, and suggested a method for preparing a standard from the perfectly fresh posterior lobes of pituitary glands. Smith and McClosky[11] very recently also presented a method for preparing a desiccated powdered standard from the fresh posterior glands.

The writer wishes at this time to confirm some of the above reports. Spaeth's objection to histamine as a standard on account of its deterioration cannot be considered a very serious or important one, as when properly made up and stored, histamine retains its activity without appreciable loss for long periods. However, it is not an ideal or perfectly satisfactory standard. The writer has prepared solutions of histamine (1-10,000), sterilized by Berkefeld filtration, placed in ampoules, and kept in an ice box for more than a year without loss in activity. However, if these ampoules are boiled for 30 minutes on three successive days, or 1 hour and one half on the same day, there is a distinct loss of potency. There seems to be a better reason for questioning its use, and that is, its different pharmacologic action, as compared with pituitary substance, on the uterine muscle. It has been the writer's experience to assay many hundreds of samples of pituitary extract with histamine and the conclusion has been reached that there is a distinct difference in their action on the uterine muscle. The sensitiveness of a uterus to pituitary extract differs from that to histamine, and the quality of its action seems unlike that of pituitary extract. The latent period of the muscle is notably shorter under histamine than under pituitary extract. Since observing this difference the writer has taken the contractions obtained during the dependable period as the conclusive reading in comparing a pituitary extract sample with histamine. In regard to potassium chloride as a standard, the writer finds a greater difference in the pharmacologic properties of potassium chloride and pituitary extract than between histamine and pituitary extract. In the use of potassium chloride, there is a greater lack of equal response during the changes of irritability of an experimental uterus. Also the sensitiveness of a uterus to pituitary extract becomes less during the experiment, and furthermore, the life or dependable period is shortened considerably as compared with histamine. In view of these findings the writer believes that histamine is a better standard than potassium chloride.

However, the use of a substance from the pituitary gland is the logical standard, as has been suggested by Burn and Dale[8] and Smith and McClosky.[11]

Three samples *D, E* and *F* were prepared according to the method of Burn and Dale[8] from three different lots of perfectly fresh posterior glands, and three samples *G, H* and *I* according to the method of Smith and McClosky.[11] Tables I and II show the comparison with a standard pituitary extract which represents 5 per cent fresh posterior lobe.

TABLE I

METHOD OF BURN AND DALE

PREPARATION NO.		STANDARD PITUITARY EXTRACT
2 c.c. D	=	1 c.c.
1.8 c.c. E	=	1 c.c.
2.1 c.c. F	=	1 c.c.

TABLE II

METHOD OF SMITH AND MCCLOSKY

PREPARATION		MG. OF FRESH POSTERIOR LOBE
1 mg. G	=	6.7 mg.
1 mg. H	=	6.6 mg.
1 mg. I	=	6.8 mg.

The above data show a remarkable consistency in the potency of different lots of pituitary glands, and either method would be satisfactory for preparing a standard for comparing or standardizing an unknown sample. There is an advantage in a standard powder, in that enough of the powder can be made at one time to last a year, while a liquid standard would have to be made more often, thus requiring considerable time and careful technic. The writer, for the past three years, has used a powder prepared from the posterior gland and has checked this against a standard histamine solution.

CONCLUSIONS

1. By using duplicate apparatus or apparatus with multiple units, it is possible to utilize more than one horn of the uterus, and to increase the number of tests of a sample in a given time.

2. A method of regulating the temperature is shown, by which it is possible to maintain a constant temperature which increases the accuracy of the oxytoxic assay.

3. It is possible to prepare a uniform desiccated powder from the pituitary gland which may be used as a standard to compare different lots of pituitary extract.

The writer is much indebted to Mr. T. Barone for the electrical work, to Mr. E. Stuart for the glass work, and to Dr. A. L. Walters and Mr. C. R. Eckler for their many valuable criticisms and suggestions.

REFERENCES

[1]Dudley, H. W.: Jour. Pharm. and Exper. Therap., 1919, xiv, 295.
[2]Dudley, H. W.: Ibid., 1923, xxi, 103.
[3]Dale, H. H., and Laidlaw, P. P.: Ibid., 1912-13, iv, 75.

[4]Roth, G. B.: Bull. Hygiene Lab. No. 100.
[5]Eckler, C. R.: JOUR. LAB. AND CLIN. MED., 1917, ii, 819.
[6]Pittinger, P. S.: Jour. Am. Pharm. Assn., 1918, vii, 512.
[7]Pittinger, P. S.: Ibid., 1922, xi, 338.
[8]Burn, J. H. and Dale, H. H.: 1922, Reports from the Dept. of Biological Standards Medical Research Council.
[9]Pittinger, P. S. and Quici, A.: Jour. Am. Pharm. Assn., xii, No. 1, 14.
[10]Kochman, M.: Ztschr. f. Physiol. Chem., cxv, 305.
[11]Smith, M. I. and McClosky, W. T.: Public Health Reports, xxxviii, No. 11, 493.
[12]Spaeth, R. A.: Bull. Hygien. Lab., No. 115.
[13]Nelson, E. E.: JOUR. LAB. AND CLIN. MED., 1923, viii, 318.

THE SIGNIFICANCE OF NEGATIVE BLOOD CULTURES DEPENDS UPON THE METHOD USED*

BY AUBREY H. STRAUS, M.A., RICHMOND, VA.

THE theme of an article appearing recently in this Journal[1] is so contrary to my experience and that of previous workers that I feel it is a duty to call attention to these differences. This article ends with the following summary:

1. "The bactericidal influence of the blood drawn for blood cultures has been overestimated.

2. "The element of chance in obtaining a positive or negative blood culture plays an important rôle.

3. "Larger quantities of blood and lower dilutions should be used in order to offset this element of chance. High dilutions of the sample of blood are not essential.

4. "At times there may be an advantage in first incubating the sample of blood for twenty-four hours before it is distributed to different nutrient media or poured on to plates."

Table I of that article contains a mass of figures gathered from various sources, but its general purport is not sufficiently clear to me to permit of discussion. The first of the above conclusions is apparently based on three experiments, in which a hemolytic streptococcus is found to multiply in defibrinated human blood, and also on a number of quotations from the work of others. Assuming the streptococcus experiments to be correct, there is only one thing proved; namely, that a hemolytic streptococcus multiplies in defibrinated human blood. Dr. Kipnis, however, concludes that "the B. typhosus, B. coli, B. mucosus capsulatus, most streptococci, staphylococci, and pneumococci, resist the bactericidal action of the blood of the host, and thus are not inhibited by any bactericidal effect of the serum, but tend to multiply." I do not feel qualified to discuss some of these organisms, but have made some study of B. typhosus and its occurrence in blood.

In the Richmond City Laboratory we have for many years examined blood

*From the Laboratories of the Richmond Health Bureau and Virginia State Board of Health.
Received for publication, October 9, 1923.

cultures as a part of our routine work.[2] Blood cultures under conditions of this sort are a very different proposition from those made in hospitals with every facility available. Simplicity was an absolute essential, and many media were tried in an endeavor to find the one best suited to our needs. A number of comparative tests were made using various media, but in every case the best results were obtained with bile (to which 1 per cent of peptone had been added.) As bile media would not grow many organisms apt to be found in the blood, such as the streptococcus, pneumococcus, etc., it was necessary to use bouillon in addition, if the cultures were to be complete. Our method has been to use a bottle of bile and a bottle of beef infusion bouillon (to which 1 per cent sodium citrate and 0.9 per cent salt are added). Each bottle contains 10 c.c. of media, to which is added a varying quantity of blood, averaging perhaps about 2 c.c. to a bottle. Table I gives the results of 822 made by this method.

TABLE I

SUMMARY OF BLOOD CULTURES

	NUMBER	PER CENT
Positive	147	18
Negative	647	79
Unsatisfactory	28	3
Total	822	100
Positive cultures as follows:		
125—Typhoid		
7—Streptococci		
5—Paratyphoid B.		
3—Pneumococci		
1—B. coli		
1—Influenza bacillus		
1—Meningococcus		
2—Unidentified bacilli, probably paratyphoid		
1—Unidentified bacillus, resembling the influenza bacillus		
1—Hemolytic, Gram-negative bacillus, unidentified		

In Table I the 125 positive typhoid cultures were all obtained from the bile. Unfortunately it is not possible to go back over our records and say exactly how many were also recovered from the bouillon. We are confident, however, that 10 per cent would be a liberal estimate. During the last two months we have had 18 positive typhoid cultures (not included in Table I) in which both bile and bouillon were inoculated. In only two of these has the typhoid bacillus grown in the bouillon. The paratyphoid B. cultures, the B. coli culture and the 2 unidentified organisms resembling paratyphoid, recorded in Table I, also failed to grow in the bouillon; on the other hand the streptococcus, pneumococcus, influenza bacillus and meningococcus grew only in the bouillon.

We occasionally find a blood which is not bactericidal, and this may account for the occasional growth of B. typhosus in the bouillon, or it may be that in some cases very little blood is used, thus giving sufficient dilution to permit growth.

We believe the above results to be due to the bactericidal effect of blood. In order to prove that blood is bactericidal for B. typhosus, experiments were conducted as shown in Table II. This table represents the average results of

10 individual experiments. The blood used in these 10 cases came from dispensary patients, two of whom gave a history of having had typhoid fever many years ago, while the other 8 gave negative histories. None of them had ever received typhoid vaccine.

These experiments were conducted by preparing a saline emulsion of 24 hour agar cultures of B. typhosus (Hopkins strain). This emulsion was standardized by density each time to approximately 1,000 million bacilli per c.c. One tenth c.c. of this emulsion was put into 10 c.c. of sterile water and 0.1 c.c. of this dilution likewise diluted into 10 c.c. of water. Of this final dilution 0.1 c.c. was used for the test adding it to 0.9 c.c. of blood or serum. This was estimated to give 1,000 colonies per plate but usually did not run this high. Some of the bloods used appeared to be more highly bactericidal than others, but each showed a strong bactericidal tendency.

TABLE II

AVERAGE RESULTS OF 10 TESTS, SHOWING THE BACTERICIDAL EFFECT OF WHOLE BLOOD AND BLOOD SERUM ON B. TYPHOSUS

BLOOD AND SERUM FROM SAME 10 PERSONS	COLONIES COUNTED ON PLATES MADE			
	IMMEDIATELY	½ HOUR	1 HOUR	2 HOURS
Whole blood defibrinated	410	113	71	17
Serum	369	73	52	9

The second conclusion was, "The element of chance in obtaining a positive or negative blood culture plays an important rôle." This is unquestionably true, but where B. typhosus and related organisms are concerned the chance of obtaining a falsely negative report is materially lessened by the use of bile media. Where bile is used, larger amounts of blood may be used without any danger of the inhibition of growth. The effect of bile in neutralizing the bactericidal properties of blood is shown in Table III.

TABLE III

AN AVERAGE OF 10 INDIVIDUAL TESTS, SHOWING THE EFFECT THAT THE ADDITION OF BILE HAS ON THE BACTERICIDAL PROPERTIES OF BLOOD

SOLUTION	COLONIES COUNTED ON PLATES MADE			
	IMMEDIATELY	½ HOUR	1 HOUR	2 HOURS
Whole Blood	720	108	31	3
Blood + 1% Bile	710	117	44	7
Blood + 2% Bile	715	152	56	13
Blood + 5% Bile	600	267	123	26
Blood + 10% Bile	580	694	708	710
Sterile water control	1300	1150	1120	1020

As shown in Table III the addition of 10 per cent bile transforms a bactericidal blood into a culture medium. While 5 per cent bile reduced the bactericidal effect of the blood, virtually no effect is noted by the addition of bile in amounts as small as either 1 or 2 per cent. In order to be certain that the decrease of typhoid bacilli was due to the action of the blood and not to physical causes a control was made with each emulsion used, adding the bacterial emulsion in the same proportion to the blood and to sterile water tubes. The

total volume used in each test was 1 c.c. and the general technic the same as described in connection with Table II.

The third conclusion, "Larger quantities of blood and lower dilutions should be used in order to offset this element of chance. High dilutions of the sample of blood are not essential," has already been sufficiently covered in the discussion of the first and second conclusions.

The fourth conclusion, "At times there may be an advantage in first incubating the sample of blood for twenty-four hours before it is distributed to different nutrient media or poured on to plates," is only proved by Dr. Kipnis for one variety of streptococcus. Our own efforts to grow the streptococcus in whole blood have been unsuccessful, but the fact that we were working with different strains might readily account for this difference.

We have found only one blood in which B. typhosus could multiply. We have found, however, that it will remain alive in clotted blood for as much as four or five days. The serum in most of these cases was strongly bactericidal, and yet the clots could be put into bile media and B. typhosus recovered. We have, as yet, no explanation for this seeming inconsistency, but very satisfactory blood cultures can be made from clots, as far as organisms of the typhoid group are concerned.

CONCLUSIONS

1. Defibrinated human blood, or the serum, is bactericidal for B. typhosus.
2. Bouillon in low dilutions is not a satisfactory medium for cultivating organisms of the typhoid-colon group from blood.
3. Satisfactory blood cultures for a large variety of organisms can be made by using two media; namely, bile for the typhoid-colon group, and beef-infusion bouillon for the streptococcus, pneumococcus, etc.

REFERENCE

[1]Kipnis: Jour. Lab. and Clin. Med., July, 1923.
[2]Straus: Am. Jour. Pub. H., Aug., 1921.

A SATISFACTORY METHOD FOR DEMONSTRATING GASTRO-INTESTINAL MOVEMENT ON THE FROG*

By H. P. Rush, M.D., Portland, Ore.

FLUOROSCOPIC observations of the frog have given us a simple yet satisfactory method for demonstrating gastrointestinal movements. Anyone who has frequently attempted to demonstrate these movements by direct observation has doubtless had the experience so nicely stated by Auer, "When the abdominal cavity is opened a hush seems to fall upon the viscera." Mention has been previously made of fluoroscopy on the frog by Cannon, although the greater part of his work seems to have been done with mammals.[1] I

*From the Hull Physiological Laboratory of the University of Chicago.
Received for publication, November 3, 1923.

believe where circumstances will not permit the training and keeping of such animals, the frog work described here will be a helpful substitute, and might be used to advantage in connection with the method previously described by Patterson.[2]

The method I have contrived is founded upon three fundamentals: (1)

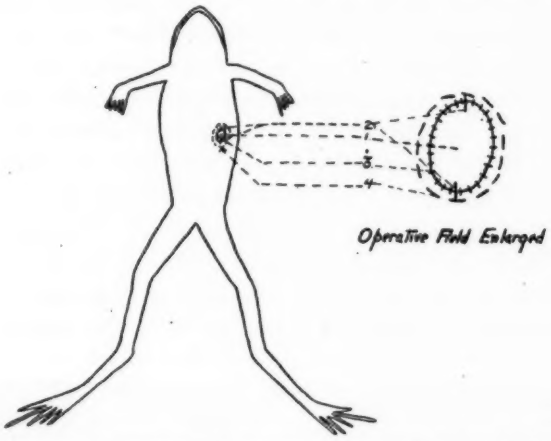

Fig. 1.—*1.* Gastrostomy opening. *2.* Stay sutures used to hold stomach wall in place while incision is made. *3.* Over and over stitch. *4.* Purse string suture, applied just before the tube is put in place.

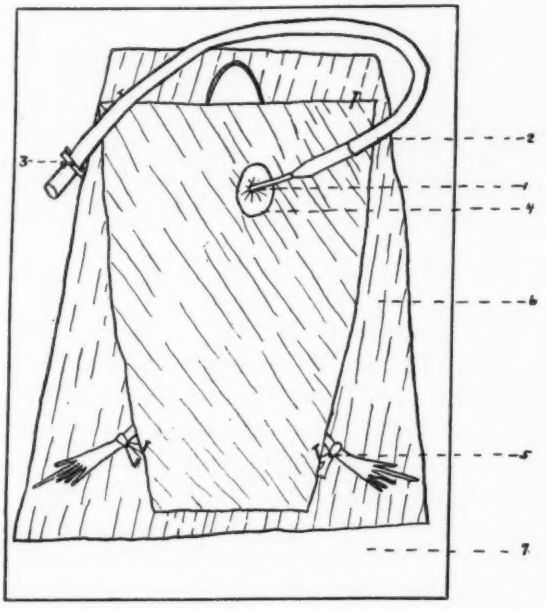

Fig. 2.—*1.* Gastrostomy with tube in place. *2.* Tube for introduction of acid. *3.* Clamp. *4.* Opening in moistened covering. *5.* Gauze lashes to hold animal in place. *6.* Moistened cotton beneath frog. *7.* Frog board.

a small x-ray unit with fluoroscope; (2) a common laboratory animal, the grass frog, care being taken to choose a large, well nourished individual; (3) a gastrostomy two or three days previous to the demonstration.

For best results the frog should be fed two or three days before the

work is to start, as most laboratory frogs are in a hibernating condition. Active digestion will thus be brought about by the time the animal is ready for observation. Live worms, flies or insects of any kind offer a good diet.

The gastrostomy, a simple operation, is a necessity for the best results, because of the easily elicited reflexes which occur when the opaque meal is given orally. Almost invariably, even though the frog has no esophagus worth mentioning, passage of a small stomach tube for introduction of the barium, will initiate vomiting and inhibition of the normal movements. The operation consists of removing a small portion of the ventral abdominal wall, measuring 1 x 0.5 cm., on the left side, starting the incision about half way down the abdomen. Care must be taken not to incise one of the parietal branches of the abdominal vein, as much hemorrhage usually results fatally within a few days. If these veins are avoided, this being easily accomplished, the operation will be practically bloodless. Gently push the left lung aside with blunt forceps and pick up the stomach, guarding against trauma. Two or three stay sutures can now be taken to hold the organ in place after

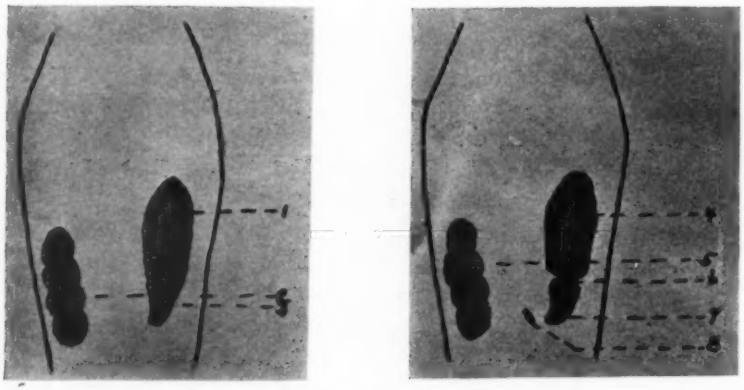

Fig. 3.—*1* and *4*. Body of stomach. *2* and *5*. Colon. *3*. Pyloric antrum. *6*. Peristaltic wave seen in pyloric region. *7*. Pylorus opening. *8*. Material moving down intestinal tract.

which it is opened by a short linear incision. I used an over and over suture around the edges, only one row being necessary for stomach wall, muscle and skin. It has been more satisfactory in my work if the stomach incision was made in the body of the stomach and not too close to the cardia. This seems to lessen inhibitory reflex stimulation. This operation need not be performed aseptically as the frog is practically immune from infection. Ether anesthesia is used for these animals, and the skin is washed well after the operation to remove any excess fumes that might still be present. The animal is allowed two or three days for the healing of the wound and then, after decerebration, a small rubber tube 0.3 cm. in diameter and about 2 cm. in length is placed in the opening and held intact by a purse string suture, the suture going through the tubing. I decerebrated by destroying all brain substance anterior to the cephalic margins of the tympanic plates. A period of thirty to sixty minutes was allowed for the animal to fully recover from shock.

The frog is placed on a frog board and tied in position. Small gauze

lashes tied as loosely as possible about the legs and these in turn pinned to the board, give less reflex disturbance than to pin the feet down directly. There should be a layer of cotton moistened with water beneath the animal and one also to cover it. These should not be allowed to dry out.

I have been using a mixture of barium sulphate and ordinary starch, the starch holding the barium in suspension. About one or two cubic centimeters are given through the tube in the gastrostomy by means of a pipette. A small rubber tube with a cannula in one end is filled with 0.1 to 0.2 per cent HCl, clamped off at the free end with hemostats and by means of the cannula connected with a tube in the gastrostomy.

The preparation is placed under the fluoroscope and the animal moved about so as to obtain the best possible view. While the filled stomach is under observation a few drops of the acid should be allowed to enter. This can be accomplished by releasing the hemostats for an instant, the tubing being raised at the same time. Within two or three minutes deep peristaltic waves will be seen passing down the wall of the stomach. From time to time the barium will be observed to pass into the duodenum. Care must be taken in this work not to over distend the stomach, as this will inhibit the movements sought. The activity of the colon may also be observed at the same time, providing a barium meal has been given the day before observing.

Frogs thus prepared have been kept in good condition for over a week, daily observations being made on them. It should be pointed out that there is a real danger with these animals of local necrosis of the area surrounded by the purse string suture. However, this does not occur for three or four days.

Fig. 1 shows the location of the incision. Fig. 2 shows the preparation ready for observation. Fig. 3 shows diagrammatically the results obtained by this method.

REFERENCES

[1]Cannon, W. B.: The Mechanical Factors of Digestion.
[2]Patterson, T. L.: JOUR. LAB. AND CLIN. MED., 1920, v, 674.
[3]Marshall, A. Milnes: The Frog; Anatomy, Histology and Embryology.

The Journal of Laboratory and Clinical Medicine

VOL. IX ST. LOUIS, MO., FEBRUARY, 1924 NO. 5

Editor-in-Chief: WARREN T. VAUGHAN, M.D.
Richmond, Va.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - CINCINNATI
PAUL G. WOOLLEY, M.D. - - DETROIT
J. J. R. MACLEOD, M.B. - - - TORONTO
ROY G. PEARCE, M.D. - - AKRON, OHIO
W. C. MACCARTY, M.D. - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - COLORADO SPRINGS
VICTOR C. MYERS, PH.D. - - NEW YORK
MARSH PITZMAN, M.D. - - - ST. LOUIS

Contents of this Journal Copyright, 1924, by The C. V. Mosby Company—All Rights Reserved
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIALS

A New Use for Epinephrine

A LONG and mysterious story was begun by Bartholomeus Eustachius when he discovered the "Glandulae renibus incumbentes" (adrenal glands) in 1563 in Rome when he was professor of anatomy at the Collegia della Sapienza. In 1849 Thomas Addison in London described the effects of disease of the suprarenal glands (Melasma suprarenale) which syndrome Trousseau later designated as "Addison's disease." The experimental proof of the accuracy of these clinical observations was first demonstrated by Brown-Séquard (1856-58). In 1894-95 Oliver and Schafer in England showed that aqueous extracts of the adrenal glands, when injected intravenously, produced a marked rise in blood pressure accompanied by slowing of the heart. Much further experimental work on these glands by Abel and Takamine led to the isolation and production in crystalline form of the active principle now known as epinephrine or adrenalin (1901). Notwithstanding these brilliant discoveries the average textbook on physiology of one or two generations ago disposed of the suprarenal glands with a line or so at most, usually—much to the satisfaction of the student—with the simple statement that "the adrenal glands have no known function."

In recent years, however, as a result of a great amount of experimental

work, and by the aid of, or perhaps often in spite of, a vast deal of speculative theorizing, sometimes, by those whom Carlson has suspected of being afflicted with "endocrinitis," more and more light is slowly being thrown on the mysteries of the adrenal glands. The mere fact that a relatively large dose of adrenalin suddenly injected into the circulation will produce a great number of very striking and spectacular effects in the body, does not by any means indicate that the adrenal glands are normally acting in such a manner as to bring about in a minor degree and in a more or less continuous fashion the results which a large intravenous injection produces. This is, perhaps, even less true than would be the suggestion that the smoking of a mild cigarette must necessarily produce in minor degree all the profoundly complex and striking results which a large dose of nicotine would bring about.

From time to time the possibility of new uses for epinephrine are being suggested. One of the most recent of these has been proposed by Douglas.[1] Since 1920 this investigator has been experimenting with epinephrine with a view to its use in preventing absorption into the systemic circulation of toxic substances produced locally in the tissues. One of the specific instances in which this method may be of service is in the treatment of burns. In a preliminary report from the surgical department of the University of Cincinnati Medical School Douglas has shown that epinephrine may very effectively prevent the absorption of solutions of strychnine (1/10 gr.) from the abraded surface of the ear of a rabbit if the area (3 sq. cm.) has previously been treated locally for a short time with adrenalin which is removed before application of the strychnine. Procaine, which was dissolved in the adrenalin solution, served not only to increase the local vasoconstricting action of the epinephrine, but also acted as a local anesthetic, and this action, in case of clinical use, would afford the patient much relief in the changing of dressings, etc. Douglas found that in case of local burns a vast absorbing area of capillaries was thrown open for the absorption of whatever soluble toxic materials might have been produced in the surface tissues as a result of their primary destruction by the burn, or as a result of their later changes or infection. Application of a solution of adrenalin and procaine to these capillary areas and their associated small arterioles and venules caused sufficient local vasoconstriction to markedly decrease the absorption from the wound. This constriction could be prolonged over extended periods by constant or intermittent local application of the drugs, the pressor action of the drugs being stronger than the vasodilator forces at work. The epinephrine tended to prevent its own absorption and destruction by its vasoconstricting action. Absorption through the lymphatics and by tissue diffusion seemed negligible.

The treatment is suggested not only for burns, but probably for localized trauma from a variety of causes and possibly for cases of localized infection, such as infected wounds, erysipelas, pleural empyema, cystitis, etc. The vasoconstriction may also allay the extensive outpouring of blood elements into the tissues and the attendant signs of inflammation.

In relation with these experiments one cannot avoid considering the use

of local anesthetic solutions containing adrenalin, when these are employed for nerve blocking procedures in connection with a general anesthetic.

These experiments tend to add further interest to the adrenal glands and their only isolated active principle.

REFERENCE

[1]Douglas, B. D.: Jour. Amer. Med. Assn., Dec. 8, 1923, lxxxi, 1937.

—*D. E. J.*

Acute Rheumatic Fever

ALTHOUGH acute inflammatory rheumatism is recognized as a distinct clinical entity with thoroughly characteristic objective symptoms indicative of acute infection, the etiologic agent has steadfastly resisted all attempts at isolation. This is particularly surprising in view of the clear-cut localization of the inflammatory process in certain organs and tissues where one would naturally feel confident of discovering the causative microorganism. As a consequence, certain observers have questioned its bacterial origin and have suggested that the disease may be due to the activity of a filterable virus; or that it may result from systemic intoxication from a germ remaining localized in some focus of infection; or again, that it may be a manifestation of some allergic or other noninfectious reaction. So far there is no more definite evidence to support these theories than exists in defense of the bacterial hypothesis. Twenty odd years ago we had hopes that the riddle would be solved by the researches of Poynton and Paine, who described the diplococcus rheumaticus as the probable cause of inflammatory rheumatism. Even they were unable to isolate this bacterium in any large proportion of cases and the work of others has not as yet substantiated their claim. Rosenow in 1914 cultivated anerobically three different strains of diplostreptococci, which he had isolated from the joints, the blood and the tonsils of rheumatic cases. On injection into rabbits, these microorganisms produced arthritis, pericarditis, and endocarditis, and one caused in addition, myocarditis and myositis.

Poynton and Paine in 1901 presented quite as detailed evidence in favor of their microorganism as has Rosenow more recently for his. They found the diplococcus variously in the blood, pericardial fluid, endocardial lesions, nodules, heart wall and throats, and on rabbit inoculation produced arthritis, synovitis, choreiform movements, valvulitis and pericarditis. The researches of Poynton and Paine and of Rosenow have not been fully confirmed, and indeed most workers have found, as emphasized by Horder, that the cardiac vegetations are free from bacteria and that blood cultures are customarily negative. Riesman in particular has argued against the streptococcus or diplostreptococcus as the primary cause, but suggests that it may serve as a secondary invader as it often does following infection with the virus of influenza. The most characteristic postmortem lesion is the presence of the Aschoff bodies in the heart muscle, but up to the present neither the streptococcus nor the diplococcus rheumaticus has been isolated from these bodies.

Whether the diplococcus of Poynton and Paine or the diplostreptococcus of Rosenow or some other as yet unrecognized germ is the specific cause of acute articular rheumatism, the fact remains that treatment as an acute infection is productive of best results and that removal of presumptive foci of infection customarily results as satisfactorily as in other infectious conditions directly attributable to focal infection.

That the recognition of the causative agent in rheumatic fever would be a tremendous step forward in the prevention of the disease is indicated by analogy with tuberculosis in which the discovery of the tubercle bacilli and the recognition of its distribution in the sputum, urine, feces, and milk, and its viability in dust, etc., was of great aid in the application of the principles of preventive medicine. The recognition of the primary cause alone will not be sufficient. We must know definitely its mode of entrance and exit from the body and the degree and manner of infectivity.

That the disease is mildly contagious has not been clearly demonstrated, but certain observations serve to corroborate this opinion. W. St. Lawrence has studied the incidence of rheumatic fever and tuberculosis among New York families. In 100 families, each containing at least one individual with acute rheumatic fever, he found that 14.8 per cent of exposed persons developed the same condition. In a similar study the percentage of familial tuberculosis was 14.6. Raven reports a small epidemic in a seaside hospital.

Assuming the disease to be infectious and perhaps contagious, and recognizing the tendency of the infecting virus toward invasion of vital tissues, paricularly the heart muscle and endocardium, what are the best means of preventing additional damage in the individual case and for safeguarding others? The presumptive focus most frequently incriminated is the tonsil. Other foci may play a part in the occasional case. Robey reports a case apparently directly attributable to an infected tooth and is of the opinion that occasionally other foci may play a part. Tonsillectomy is generally considered an important prophylactic procedure, even after the development of an attack. Janeway had doubts as to its value after the development of an acute attack. Robey feels that the removal of diseased tonsils is clearly indicated even if the heart has already become involved. St. Lawrence in a study of 94 children, concluded that tonsillectomy seemed to be the most important measure at present available for the prevention of acute rheumatic fever and allied rheumatic affections. Lambert concluded that tonsillectomy played an important part in the reduction of acute arthritis at Bellevue Hospital. Lillie and Lyons found that operation resulted in 79 per cent of improvement among 200 tonsillectomies for myositis and arthritis. Starling is convinced that the tonsils are the main portal of entry and favors complete enucleation thereof. He believes that an acute attack may be aborted and further infection prevented by this procedure. Hunt and Osman report a study of 144 patients, among whom those submitted to tonsillectomy showed a greater percentage of relapse than those not operated upon.

The consensus of opinion favors tonsillectomy where the tonsils are obviously diseased. Robey points out that a small fibrosed tonsil or a tonsil shriv-

elled by x-ray is often more dangerous than a large, obviously infected tonsil with patent crypts. Particularly dangerous are those tonsils which have been partially removed with the tonsillotome.

The mechanism of relapse or reinfection in those cases in which cardiac involvement has been established is not clearly understood. It is uncertain whether in endocarditis for example, the infection may be completely overcome with a resulting predisposition toward reinfection in the injured valves; or whether the causative agent has remained dormant in the tissues of the valve. Myocarditis customarily accompanies the endocarditis and until recently too little emphasis has been placed upon this latter complication.

It is as difficult to decide when the heart has become involved in a rheumatic process as it is to make certain when the active cardiac process has been completely arrested by treatment. The accumulating evidence, however, clearly indicates that cardiac invasion occurs early in the disease.

Pitcairn at St. Bartholomew's Hospital in 1788 was apparently the first to discuss a relationship between acute articular rheumatism and subsequent cardiac disease. Little further progress was made until after the introduction of auscultatory methods, when Hope in 1832 discussed rheumatic fever as one of the causes of inflammation of the lining membrane of the heart. It was not until Aschoff and Geipel demonstrated the familiar specific lesion that a definite relationship was proved. The presence and extent of cardiac involvement has been judged by the occurrence and character of cardiac murmurs, tachycardia, cardiac enlargement, and the patient's general condition, such as the degree of anemia. Some murmurs undoubtedly occur in the absence of cardiac involvement and often there is, without doubt, still some activity in the endocardium and especially the myocardium after murmurs have entirely disappeared. The changing character of the cardiac signs as observed on auscultation is usually an indication of persistent activity.

Cohn and Swift have completed a study of 37 patients suffering with rheumatic fever, 17 in the first attack, 12 in a subsequent attack and 8 in whom cardiac pathology had already been recognized clinically. The chief object of the study was to recognize electrocardiographically early evidences of cardiac involvement. They found such changes in all but two cases. The evidence was of three types. First, increased or delayed conduction of the impulse from the auricle to the ventricle usually without frank heart block; second, alteration in the ventricular complex of the electrocardiogram affecting the QRS complex, or the interval between the R wave and the T wave, or again, the character of the T wave itself; and third, the occurrence of various types of arrhythmia. These abnormalities are not confined to rheumatic fever but have been observed elsewhere. Thus, increase in the conduction time has been observed in influenza, bronchopneumonia, diphtheria and typhoid fever. But the phenomena are independent of the degree of pyrexia and although they do not occur in acute articular rheumatism alone, they are clearly indicative of myocardial involvement. They were not usually permanent.

In view of the early and frequent involvement of the endocardium and myocardium all are agreed as to the desirability of prolonged supervision of

the patient's convalescence. Raven insists that the after care of the rheumatic child should be as prolonged as and of very much the same character as that for pulmonary tuberculosis. He recommends complete rest in bed as long as there is any indication that the disease is still active. Milk is used abundantly and rest is secured preferably in seaside convalescent homes. Treatment often covers a period of months instead of weeks, quite a contrast to the older routine as described by Robey. "Sir Andrew Clark, when once asked what he considered the best treatment for rheumatism, replied 'Six weeks.' We generally gave our patients six weeks, at the end of which time they were ready to leave the hospital. We bade them goodbye and stood ready to welcome them upon their return, which was sure to occur in numerous instances."

Although rheumatic fever appears to be less common than in the past, recrudescences almost of epidemic character occur from time to time and the disease is sufficiently prevalent to warrant continuation of intensive study. Such an opportunity exists in the seaside hospitals and convalescent homes described by Raven and it is to be hoped that before long distinct advances may be made in our knowledge of the etiology of this disease.

REFERENCES

Raven: Lancet, London, Dec. 8, 1923, ccv, 1227.
Robey: Boston Med. and Surg. Jour., Jan. 17, 1924, cxc, 89.
Cohn and Swift: Jour. Exper. Med., Jan., 1924, xxxix, 1.

—*W. T. V.*

Conference of Maritime Quarantine Authorities of the West Coast of South America

DOCTOR BELISARIO PORRAS, the President of the Republic of Panama, has called a conference to meet in Panama, R. P., February 25-29, 1924, for the purpose of considering the international standardization of maritime quarantine on the west coast of South America and the prevention of international spread of communicable disease in that littoral.

The Conference, which is under the immediate direction of the Honorable Colonel Juan Antonio Jimenoz, Secretary of Fomento y Obras Publicas, will hold formal discussions each morning, at which will be discussed questions bearing upon maritime quarantine regulations; the methods, periodicity, and certification of ship fumigation; uniform quarantine declarations and uniform bills of health. Afternoons will be devoted to practical demonstrations of public health and hospital methods. Clinics will be held at Santo Tomas, Ancon, Corozal and Palo Seco hospitals. There will also be demonstrations in municipal hygiene, garbage collection and destruction, public markets and refrigerating plants at Panama and Colon, R. P. The Conference will inspect the antimalarial work which is being done by the Health Department of the Panama Canal and will make a study of ship fumigation with cyanogen chloride. The water works purification plant will be inspected, and there will be a demonstration in public health laboratory methods at the laboratory of the Health

Department of the Panama Canal. The Instituto Nacional and the Normal School will give an exposition of methods of teaching public health to children, and the results in improved sanitation coincident upon the installation of the national road system of the Panama Government will be shown. The Medical Association of the Isthmian Canal Zone will hold a special meeting at the Santo Tomas Hospital for the Conference. There will be a visit to the site of the Gorgas Memorial Institute and it is believed that this will constitute one of the outstanding features of this international meeting.

There will be many social functions for the delegates, among the most notable of which will be dinners given by the President of Panama and the Herrick Clinic, and luncheons tendered the Conference by the Rotary Clubs of Colon and Panama and the Panama and Colon Associations of Commerce.

The Secretary-General of the Conference is Surgeon William Colby Rucker, U.S.P.H.S., Chief Quarantine Officer of the Panama Canal. Physicians, surgeons and public health workers visiting the Isthmus of Panama at the time of the Conference will be welcomed.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Diathermy and Its Application to Pneumonia**

ELECTROTHERAPEUSIS, without doubt, has a place in the modern treatment of disease. It is difficult however, as yet, to determine just what position it holds in the scale of relative values. As we come to know more of its mechanism and biologic action we may develop a greater field of usefulness for this procedure. If we class x-ray therapy under electrotherapeutics, we may say that tremendous strides have already been made. With regard to diathermy itself its definitive value still hangs in the balance. The raising of the internal temperature of organs and tissues by the application of electrodes to body surfaces doubtless is beneficial at times. The author does not confine himself to the results of diathermy in the treatment of pneumonia but covers the whole field of diathermy in the various diseases. The major portion of the book deals with the technic of treatment. The results thereof are detailed only with regard to pneumonia. Here, as in any treatment of lobar pneumonia, the innate variability of the course of the individual case renders deductions from case reports untrustworthy. When, after serum therapy has been in use for nearly a decade and statistics have been compiled on thousands of cases, many still question its actual value, we

*Diathermy and its Application to Pneumonia. By Harry Eaton Stewart, M.D., Attending Specialist in Physiotherapy, U. S. Marine Hospitals, N. Y.; Consultant in Physiotherapy, U. S. V. B. Hospital, New Haven, Conn.; Director, New Haven School of Physiotherapy, Office of the Surgeon General, U. S. Army, and Supervisor of Physiotherapy, Bureau of U. S. Public Health Service, Washington. Forty-five illustrations and fifteen charts. Cloth. Price, $3.00. Pp. 210. Paul B. Hoeber, Inc., New York, 1923.

cannot expect conclusive evidence from the small number of pneumonia patients treated to date with diathermy. The case reports appearing in this volume are sufficiently detailed that one may draw conclusions with regard to the individual case. In certain ones the application of this therapeutic method appears to have been followed within a reasonable period by amelioration of symptoms and by subsidence of the acute process. Perhaps the majority of cases reported, went on through the customary seven days before complete defervescence, but the temperature fell usually by lysis which began earlier in the disease. The author reports decided subjective relief, with lessened pleuritic pain and diminution of cyanosis. The mortality in his series of carefully controlled hospital cases was 19.4 per cent.

Some experimental work is briefly described in explanation of the mechanism of diathermy.

---

*The Heart**

DR. NEUHOF has produced an unusually complete volume dealing with the normal heart and with cardiac physiology and pathology. Instead of devoting entirely separate sections to the special forms of laboratory examination such as electrocardiography, polygraphy, teleroentgenography and orthodiascopy, he has distributed this information through the book in the discussion of the different pathologic conditions. This is the logical procedure since, by now, all who are especially interested in cardiologic study are well acquainted with the mechanics and the general principles of these special precision instruments. However, for those who have been unable to acquire such familiarity, the author has incorporated technical chapters dealing with the use of these instruments. He stresses the observation that, once having acquired through graphic methods an understanding of the cardiac arrhythmias, these mechanical instruments are seldom necessary in routine clinical diagnosis. Starting with the embryology and the gross and microscopic anatomy of the heart, the book develops logically through a study of the fetal circulation and cardiac physiology to a consideration of the various aspects of disease as it appears in this organ. Vascular disease is touched upon in so far as it relates to disease of the heart itself. Functional efficiency tests are discussed and the author concludes, as do most other cardiologists, that the best indication of functional efficiency is that obtained by simple forms of exercise and by the patient's reaction to the stress of daily life. Cardiologic reactions in disease of other portions of the body receive detailed consideration.

The second portion of the book contains a series of cardiac clinics in which facts previously outlined are brought into actual clinical experience.

Illustrations are plentiful and clear.

---

*The Heart. By Dr. Selian Neuhof, B.S., M.D., Visiting Physician, Central and Neurological Hospital; Consulting Cardiologist, Broad Street Hospital; Associate Attending Physician, Lebanon Hospital; Former Clinical Professor of Cardiology, Fordham University, New York. Cloth. Price $10.00. Pp. 701. 300 illustrations. P. Blakiston's Son & Co., Phila., 1923.

*Human Protozoology**

THE study of protozoa has been carried on in various unrelated subdivisions of the biologic and medical sciences but there are few works in which the resulting widely disseminated information has been brought together and correlated. The authors have done this in the volume under consideration. While attention is directed primarily to the protozoa infecting man, for the sake of completeness other related protozoa are incorporated in the discussion. Much of our knowledge of human protozoology has been acquired since the beginning of the World War and this newer work has until now been available only in individual monographs.

The text is very complete and is profusely illustrated with very clear drawings and photographs. The sections devoted to amebae, trypanosomes and flagellates in general, and to malaria plasmodia should be of genuine interest not only to students of parasitology but also to clinicians in general. The description of the morphology and life cycle of the malarial parasite is the most concise and clearly written of any with which the reviewer is acquainted.

A special section is devoted to methods of examination for the intestinal protozoa. The bibliography is classified.

*Human Protozoology. By Robert W. Hegner, Ph.D., Professor of Protozoology and William H. Taliaferro, Ph.D., Associate Professor of Protozoology in the School of Hygiene and Public Health of the Johns Hopkins University. Cloth. Pp. 597. Illustrated. The MacMillan Co., New York, 1924.

The American Society of Clinical Pathologists

The American Society of Clinical Pathologists, to which the profession is looking for demonstrations of the actual value of well trained laboratory experts to the practice of clinical medicine, will hold its next meeting June 5-7, 1924, in Rochester, Minn.—Wm. Carpenter MacCarty, President.